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INDEXING PROCEDURES FOR 15 VIRUS DISEASES OF CITRUS TREES

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INDEXING PROCEDURES FOR 15 VIRUS DISEASES OF CITRUS TREES

Prepared by the Committee on Indexing Procedures, Diagnosis, and Nomenclature, Third Conference of International Organization of Citrus Virologists, Campinas and São Paulo, Brazil, 1963

Committee Members

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Contents

Italic numbers in parentheses refer to Literature Cited at the end of each section.

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Indexing Procedures For 15 Virus Diseases Of Citrus Trees

INTRODUCTION

By J. F. L. Childs

Recognized virus diseases of citrus trees are so numerous and the literature is so voluminous that selecting an appropriate indexing procedure is often a problem. Information on the preferred test plant for a certain virus, age of the test plant for best expression of symptoms, inoculation methods, incubation period, and the effect of environment and of other viruses can be of critical importance in testing for citrus viruses. For example, Rangpur lime is a good test plant for exocortis virus in Brazil and California but poor in Florida. Psorosis leaf symptoms are difficult to obtain in the State of Bahia, Brazil. In Surinam, tristeza symptoms can be obtained reliably on Key lime test plants only in an air-conditioned greenhouse. Because of this situation, publication of a handbook on indexing procedures for virus diseases of citrus was recommended by the Committee on Indexing Procedures, Diagnosis, and Nomenclature of the International Organization of Citrus Virologists at the Third Conference held at Campinas and São Paulo, Brazil, in 1963.

The Committee agreed that 15 viruses (the number depends on whether certain closely related viruses such as psorosis A and concave gum are grouped together) are sufficiently well established that procedures for indexing them should be presented. Committees of from one to four people were selected to describe the procedures required for the various viruses. Some changes have occurred in these committees. Gaetano Ruggieri died shortly after preparing a brief note on impietratura. A. P. D. McClean retired from active research and declined to prepare a formal paper on vein enation and woody gall for lack of time. We hoped to have a paper by H. Schneider explaining his histological method of diagnosing tristeza infection of sour orange (*Citrus aurantium* L.) rooted citrus trees, but because of other commitments he was unable to prepare one. L. C. Knorr, who was unable to attend the Conference, suggested that podagra should be included, but that index tests for fovea were as yet without conclusive results.

Citrus viruses occur throughout the citrus-growing districts of the world and most often in old-line varieties propagated vegetatively for many years. As a result, citrus budwood programs were set up to supply virus-free budwood to nurserymen and growers—first, the psorosis-free budwood program of California and later, one in Texas. Programs to supply budwood free of several viruses were subsequently started in Florida, California, Egypt, Brazil, and elsewhere. It is now widely recognized that most old-line citrus trees carry several viruses and that trees with a single virus are as rare as those without any. Thus, the most promising sources of virus-free citrus budwood are nucellar seedlings of the old-line varieties. Since psorosis virus was found to be seed transmitted by Carrizo citrange, one cannot assume that all citrus seedlings are automatically virus free. They must be tested for viruses at the same time that they are tested for trueness to type, productiveness, and vigor.

A minimum indexing program that covers the bare essentials is outlined here as an aid to those undertaking such a project for the first time. Seedling plants are usually employed for citrus virus indexing and seeds of the required varieties must be obtained locally or imported. Ordering large quantities of seed that cannot be used within a year is wasteful and a hardship to others who require seeds. A few hundred seeds of each of the four or five index varieties are adequate for the first year of operation. Additional seeds may be ordered as the program warrants it. Kept in a humid atmosphere at around 10° C., citrus seeds survive for 12 to 15 months with little loss in germination, but fresh seeds should be obtained each year if possible.

The seeds required are as follows: (1) West Indian lime (*Citrus aurantifolia* (Christm.) Swing.), also known as Mexican lime, Key lime, and baladi lime, for detecting tristeza virus; (2) Labsweet or Pineapple sweet orange (*C. sinensis* (L.) Osbeck), bitter sweet sour orange (*C. aurantium* L.), and Willowleaf mandarin (*C. reticulata* Blanco); or King mandarin (*C. sinensis* × *C. reticulata*) for detecting psorosis A, concave gum, and blind pocket forms of psorosis; (3) Arizona 861 or U.S.D.C.S. 60–13 selections of Etrog citron (*C. medica* L. var. *ethrog* Engl.) and Rangpur lime (*C. reticulata* var. *austera* hybrid) for exocortis virus; and (4) Orlando tangelo (*C. paradisi* Macf. × *C. reticulata* Blanco) for cachexia (xyloporosis) virus.

Pure cultures of tristeza, psorosis, exocortis, and cachexia (xyloporosis) should be available in the greenhouse for comparison. The tristeza isolate should be an insect-transmitted strain and the psorosis isolate should be a seed-transmitted strain. With exocortis and cachexia (xyloporosis), the best budwood is from trees that were indexed and found negative for other viruses. Such budwood may be obtained through the California and Florida citrus budwood programs and possibly elsewhere.

When indexing bearing seedling trees for the purpose of selecting virus-free individuals for budwood, it is of course impractical to index for all the known citrus viruses. However, if seedling trees are indexed and found free of tristeza, psorosis, exocortis, and cachexia, one can usually assume that other viruses are likewise absent. In tristeza-infested areas tristeza-free trees may be rare or nonexistent. In that case the only choice is to select trees infected with a mild strain of tristeza.

Virus-free budwood programs should be planned to fit the conditions of the area involved; namely, the citrus varieties grown and the rootstocks adapted to local soil and water conditions. For example, trifoliate orange (*Poncirus trifoliata* (L.) Raf.), an excellent rootstock, is not suited to alkaline or salty soils. The basic indexing procedures are presented here and can be selected or rejected according to the program chosen.

Including several uninoculated plants in an experiment on indexing is not entirely adequate as a check or control. In testing a candidate tree for the presence of a virus, one should duplicate the indexing procedure with a pure culture of the suspected virus, and inoculation of test plants with the pure culture should be run concurrently with tests of the candidate plant. This aspect of indexing is most important because the symptoms developed under local conditions of light and temperature may not be exactly like those described from other localities and the test plants used may not be exactly like those used by the original investigator.

The nomenclature of citrus species is of considerable importance to the investigator attempting to repeat the experiments of others. Several systems have been proposed, principally those of W. T. Swingle, T. Tanaka, and R. W. Hodgson. Swingle recognized 16 species of citrus, Tanaka 150, and Hodgson 39. Some virologists use one system and others another. This leads to confusion, because it is not always apparent which system is being used. Also an author may use one name from one system and another name from another system in the same publication. In an attempt to establish a degree of order under these conditions, a list of species names has been included at the end of this handbook. Prepared by P. C. Reece, it is essentially a reference list correlating the names used by Swingle and Tanaka.

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[1] Retired.

[2] Deceased.

PSOROSIS A, BLIND POCKET, CONCAVE GUM, CRINKLY LEAF, AND INFECTIOUS VARIEGATION

By J. M. WALLACE [1]

The diseases psorosis A, blind pocket, concave gum, crinkly leaf, and infectious variegation are considered here as a group, because they have certain common or overlapping characteristics. Infectious variegation was formerly regarded as directly related to the psorosis group, but the fact that it has been mechanically transmitted and the others have not raises a question regarding such a close relationship. However, for brevity they are discussed here as a group.

For the purposes of certifying virus-free sources of budwood, it is not essential to identify the particular virus of the psorosis group that may be present in the tree under test. Thus, short-term indexing on seedling trees will often identify concave gum, crinkly leaf, and infectious variegation, but in order to distinguish between psorosis A and blind pocket, the indicator plants must be grown for several years until bark lesions and concavities, respectively, develop.

Studies by C. N. Roistacher [2] disclosed what appear to be mild strains of concave gum virus, which rarely if ever cause zonate leaf patterns on sweet orange. Also, some investigations in Australia by Lilian R. Fraser and in California by the author have revealed sources or strains of crinkly leaf virus that do not cause typical psorosis young-leaf flecking except possibly on certain citrus varieties or selections and only under certain environmental conditions. These findings are taken into consideration in the indexing procedures recommended in this handbook for this group of diseases.

Indexing should be done in glasshouses or at least in screenhouses where the plants can be maintained in pots on benches. This facilitates care of plants, inoculation, and examination for symptoms. Symptoms may not develop well during the hot season when the temperature cannot be partially controlled. The individual investigator should determine the times of the year when symptom expression is good.

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[1] This section was reviewed by E. C. Calavan, J. Bové, Lilian R. Fraser, and T. J. Grant.

[2] University of California, Riverside, Calif., U.S.A.

INDICATORS

Indexing for the viruses that cause these five diseases may be done with seedlings of sweet orange (*Citrus sinensis* (L.) Osbeck), lemon (*C. limon* (L.) Burm. f.), and mandarin (*C. reticulata* Blanco). Studies in California by Roistacher and Nauer [3] showed that some sweet orange varieties were better indicators of psorosis A and ordinary concave gum than others. Among the named sweet orange selections that proved to be good indicators and that are fairly well known are Pineapple, Madam Vinous, Parson Brown, Bessie, Indian River, and Koethen. [4]

Other sweet oranges will serve as indicators of psorosis A, blind pocket, and concave gum. In locations where the above selections are not available, local varieties should be tested to select a useful indicator from which seeds can be obtained.

In California the sour lemon varieties, Eureka and Lisbon, are used as indicators of crinkly leaf and infectious variegation. Other kinds of lemon may serve equally as well. If lemon seeds are not available, sour orange (*C. aurantium* L.) can be substituted, since this species develops fairly good symptoms of crinkly leaf and very good symptoms of infectious variegation.

Mandarin varieties or mandarin hybrids should be included, because they react to a virus (possibly an avirulent strain of concave gum virus) that causes faint oak leaf or zonate patterns on mandarin but not on sweet orange seedlings. Roistacher found this symptom much more often on Kara and Dancy mandarins and Dweet tangor (Dancy (*C. reticulata* Blanco) × Mediterranean sweet orange) than on other mandarin varieties, such as Batangas, Honey, Kinnow, Ponkan, and Willowleaf. King mandarin was a good indicator of this virus if the inoculated plants were provided with supplemental lighting. During the winter 5 hours of additional artificial light improved symptom expression also on Kara, Dancy, and Dweet.

SYMPTOMS

It has been assumed that vein flecking on young leaves is a common symptom of these five diseases, and this was the basis for their original classification as members of the psorosis group by H. S. Fawcett.[5] There still seems insufficient reason to change this classification, although recent studies have disclosed strains of these viruses that do not produce the young-leaf flecking symptom as originally reported. Studies in California indicate that this young-leaf symptom occurs regularly on trees infected separately with the viruses of psorosis A, blind pocket, or concave gum. Roistacher found that mild strains of concave gum virus caused some flecking or vein clearing on sweet orange, even though they produced no oak leaf pattern on this species.

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[3] See footnote 2.

[4] "Lab-sweet," a pineapple-type sweet orange, has given excellent leaf symptoms of psorosis under greenhouse conditions in Florida (J. F. L. Childs).

[5] Deceased, formerly at University of California, Riverside, Calif., U.S.A.

The vein flecking and vein clearing typical of psorosis on sweet orange and the oak leaf pattern of concave gum are shown in figure 1. Actually the flecking symptom is a slight vein banding,

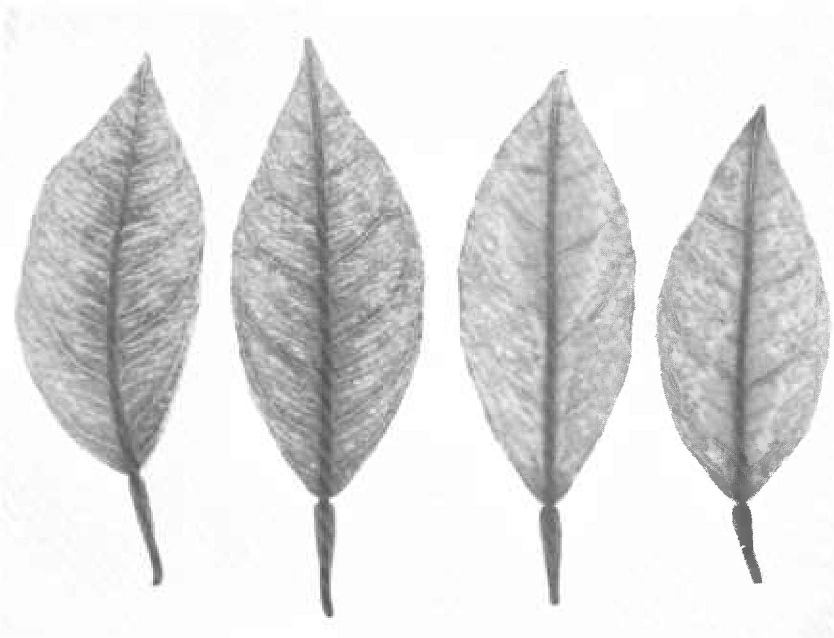

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FIGURE 1.—Young-leaf symptoms of psorosis A and concave gum on sweet orange: *Left*, two leaves with vein flecking of psorosis A; *right*, two leaves with zonate oak leaf patterns along midrib and with clearing and banding of veins on other parts.

since the lighter colored area involves some of the leaf tissues closely adjacent to the veins. The clearing is not so distinct or so narrowly restricted to the veins as that caused by tristeza. Flecking may be general over leaves or only on part of the leaf blade. Also, the flecks may coalesce so that individual flecks are not apparent and the entire area of the leaf is light in color. Thus, there may be a range of leaf effects on sweet orange, and on lemon particularly there may be circular spots or irregularly shaped chlorotic areas. The young-leaf symptoms disappear as the leaves mature and harden.

If the common strains of concave gum virus are present in the inoculum, some of the leaves of sweet orange should show the oak leaf pattern. These patterns may be visible along the sides of the midrib, over the entire length of the leaves, or only in localized areas. Other parts of the leaves show the characteristic vein flecking of psorosis. Under greenhouse conditions the patterns may not be present during all growth flushes. In California, concave gum-infected field trees display strong oak leaf patterns during the spring growth flush, but for the most part these trees show only the psorosis A type of leaf flecking during the summer and fall flushes. If the suspected mild concave gum virus is present, it should be revealed on the mandarin indicators already mentioned. Zonate or oak leaf patterns also tend to disappear as the leaves mature, but sometime faint traces of this symptom can be seen on old leaves.

With certain sources of virus, the first visible effect after inoculation is a shock reaction. Newly formed leaves become chlorotic and drop off, and the shoots that develop after the seedlings are decapitated become necrotic (fig. 2). The new leaves are usually

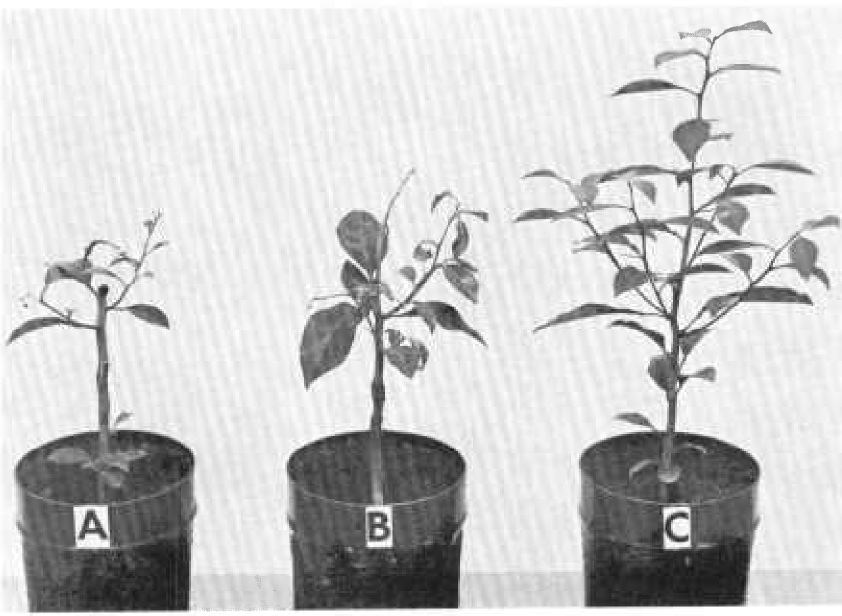

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FIGURE 2.—Psorosis shock effect on sweet orange seedlings: *A* and *B*, Shedding of newly formed leaves and terminal necrosis of soft stems; *C*, healthy, noninoculated check plant.

shed before they can develop vein flecking. Later a new cycle of growth appears with young leaves, some of which usually display psorosis leaf symptoms. Indications are that the psorosis A, blind pocket, and infectious variegation viruses can induce the shock symptoms, but there may be strains of these viruses that do not cause this symptom. Concave gum and crinkly leaf viruses apparently do not induce the shock reaction, although a combination of either or both of these two with any of the other three will cause shock.

The reaction of seedlings infected with blind pocket virus is much like those infected with psorosis A. Usually there is a severe shock effect on the first cycle of growth after infection, followed by the appearance of vein flecking on the young leaves of succeeding flushes of growth.

Unmixed with other psorosis viruses, crinkly leaf and infectious variegation viruses seldom induced strong vein flecking. On inoculated seedlings, the first flush of growth shows slight leaf distortion and blotching of leaves and usually there is leaf shedding and shock symptoms from infectious variegation. Leaves formed later commonly show pinpoint spotting, as illustrated in figure 3.

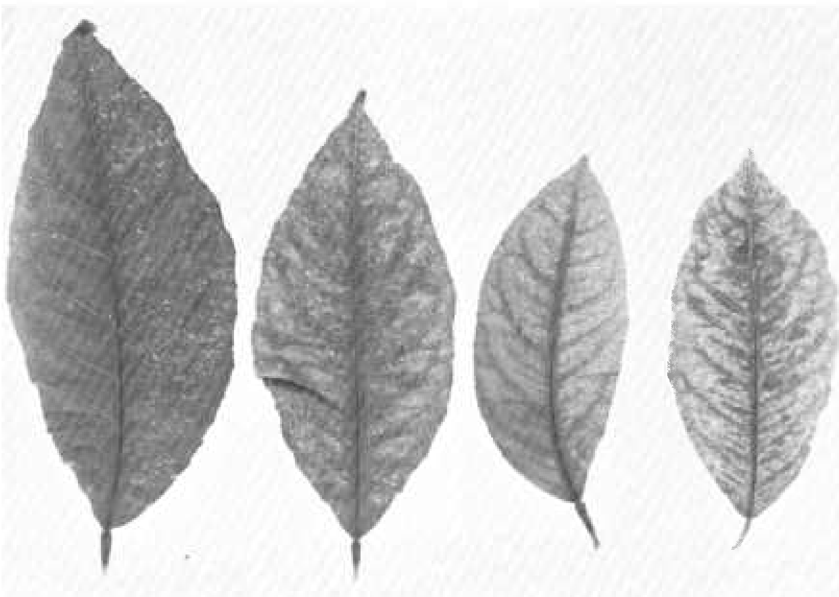

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FIGURE 3.—Pinpoint spotting of crinkly leaf and of infectious variegation on Eureka lemon leaves.

This spotting develops on immature leaves and persists. The crinkle effect develops as the leaves mature and forms a persistent symptom, as shown in figure 4. A similar type of crinkle, often associated with infectious variegation, also causes severe leaf distortion accompanied sometimes by yellowish or whitish blotchy patterns. These patterns are called the variegation effect (fig. 5).

Cross-protection reactions and the existence of sources or strains of virus that induce symptom effects intermediate between crinkly leaf and severe infectious variegation suggest a close relationship between these two viruses. Plants with infectious variegation sometimes produce recovery shoots that show only crinkly leaf and pinpoint spotting.

INOCULATION METHODS

In growing seedlings for virus indexing, the practice in California is to germinate the seeds in flats and to transplant the small

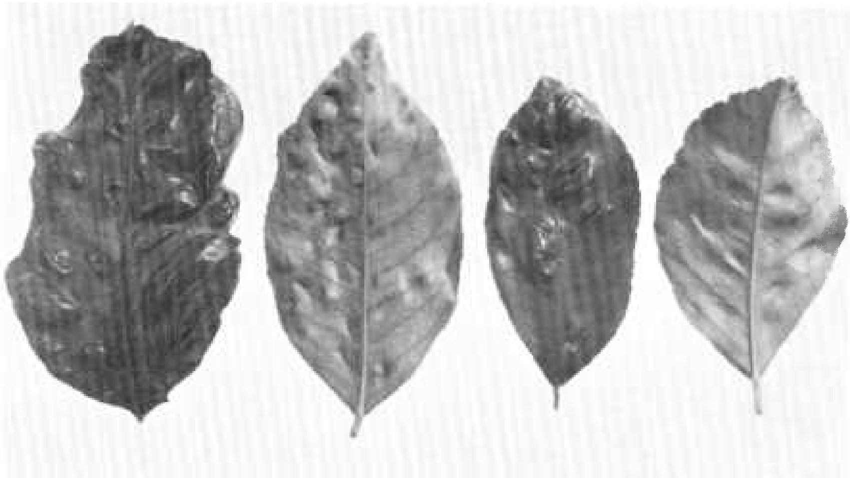

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FIGURE 4.—Persistent symptoms of crinkly leaf on mature leaves of Eureka lemon.

seedlings to pots or cans, one or two plants per container, depending on its size. Seedlings transplanted when 4 to 6 inches tall become established faster than larger transplants. A good soil mix that encourages vigorous growth should be used. The use of sterilized or fumigated soil avoids trouble from root-rotting fungi. [6]

Tissue-graft inoculations are made by means of small twig side grafts, leaf patches, bark patches, or T-buds. All types of tissue give good results, but twig grafting has three advantages. First, side grafts can be made on seedlings when they are very small. Second, one can readily determine whether the inoculum piece united with the seedling and survived, without removing the grafting tape. A third possible advantage is that more virus-bearing tissue is used. At inoculation time, the seedlings are cut back at a point two or three buds above the inoculum. When leaves are present on the stem of the seedlings, it is advisable to leave them because they offset some of the effects of topping.

Leaf patches consist of narrow rectangular pieces of the leaf blade, and bark patches are similarly shaped pieces of tissue taken preferably from small limbs or twigs with moderately thin bark. It is a good practice to clean the leaves and twigs from which the inoculum tissue is to be taken. Normally, washing and wiping are sufficient, but should one consistently find a considerable amount of decay of these tissues on the inoculated seedling, the sources of inoculum should be cleaned with alcohol or mercuric chloride (1–1,000), washed in water, and wiped dry before the patches are

[6] In Florida we find vermiculite (expanded mica chips) superior for germinating seeds, and with our organic type soil, sterilizing often results in poorer plant growth than unsterilized soil (J. F. L. Childs).

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FIGURE 5.—Chlorosis and leaf distortion on Eureka lemon infected with virus of infectious variegation.

cut out. The patches are then inserted under bark flaps made by peeling the bark downward from one horizontal and two vertical cuts and then wrapped in place with suitable grafting tape [7] or with rubberbands.

When buds are used as inoculum, they are inserted in T-cuts as in propagating citrus. If the index seedling is large enough, it is advisable to put in two pieces of inoculum. These may be the same or different; for example, one twig graft and one leaf patch. The number of test plants to be included will depend somewhat on availability of plants and the number of indicator varieties to be used. If a single indicator variety is used, sweet orange for example, at least eight plants should be inoculated and there should be two or more noninoculated controls. If plants of sweet orange, mandarin, and sour lemon are included, four inoculated and two controls of each variety should be sufficient. If the test plants are large enough, using two pieces of inoculum taken from different positions on the source tree will improve the test.

READING SYMPTOMS

If inoculation causes the shock reaction, the first symptoms are twisting and bending of the newly developing axillary shoots.

[7] We have found vinyl plastic tape of 0.0035-inch (3.5 mil) thickness superior to other tapes (J. F. L. Childs).

These symptoms are followed by leaf shedding and varying degrees of necrosis of the soft stems.

If new growth develops without the shock reaction, the leaves should be examined regularly for vein flecking or other effects. The leaves should be viewed while shaded and with the light passing through them. Faint leaf flecks may not be visible except under certain angles of lighting. Examinations should be made at 3- to 4-day intervals at least. If no symptoms are found within 6 weeks after inoculation, the plants should be cut back again and examined throughout a second cycle of leaf growth. The absence of symptoms from all indicator varieties throughout two growth flushes is reasonably good assurance that the source of inoculum was free of the viruses in question. If facilities are available and it is thought desirable, the plants can be overwintered in a screenhouse and then cut back in the spring for further study. This procedure sometimes reveals some concave gum-type symptoms that do not appear on plants maintained at all times in greenhouses.

Plants infected with crinkly leaf virus may show slight or no symptoms on the young leaves, but occasionally some faint vein flecking is present in one or more leaves of sweet orange and certain other kinds of citrus. This symptom may sometimes be evident for only 2 to 3 days. As the leaves mature, the crinkling develops on some leaves. On lemon the leaves commonly show pinpoint spotting (fig. 3). If the infectious variegation virus is present with the crinkly leaf virus, there is shock. Leaves that do not drop are chlorotic or blotched and distorted. Later growth is severely affected, showing the symptoms already described for infectious variegation.

The presence of other commonly occurring citrus viruses in mixture with the psorosis viruses seems to have no appreciable effect on the symptoms of the latter, although it is not known if this is always the case. Ideally, for budwood selection, all sources of budwood should be rejected if inoculations from them give any indication of virus infection, even though the virus or viruses present cannot be specifically diagnosed. However, in some countries the tristeza virus may be present in all available citrus budwood. Likewise, where the vein enation (woody gall) virus spreads naturally, it may be of little value to start with citrus trees that are free of this virus.

Regardless of whether or not virus-free budwood is used for propagation of new trees, diseases that spread naturally must be controlled by using tolerant or resistant rootstocks. Obviously it is wise to avoid propagation from trees carrying viruses such as tristeza and vein enation if the resulting trees are to be planted in areas where these viruses are not present.

Noninfectious Psorosislike Leaf Effects

In a study of citrus virus diseases in California, some noninfectious leaf patterns have been encountered that should not be confused with symptoms of psorosis. It seems advisable to describe the patterns so that investigators will be aware of them.

Seedlings of Eureka lemon grown in the greenhouse sometimes display the leaf-spotting effects shown in figure 6. Whether this symptom is caused by genetic or environmental factors is not known, but at times it closely resembles psorosis leaf spotting

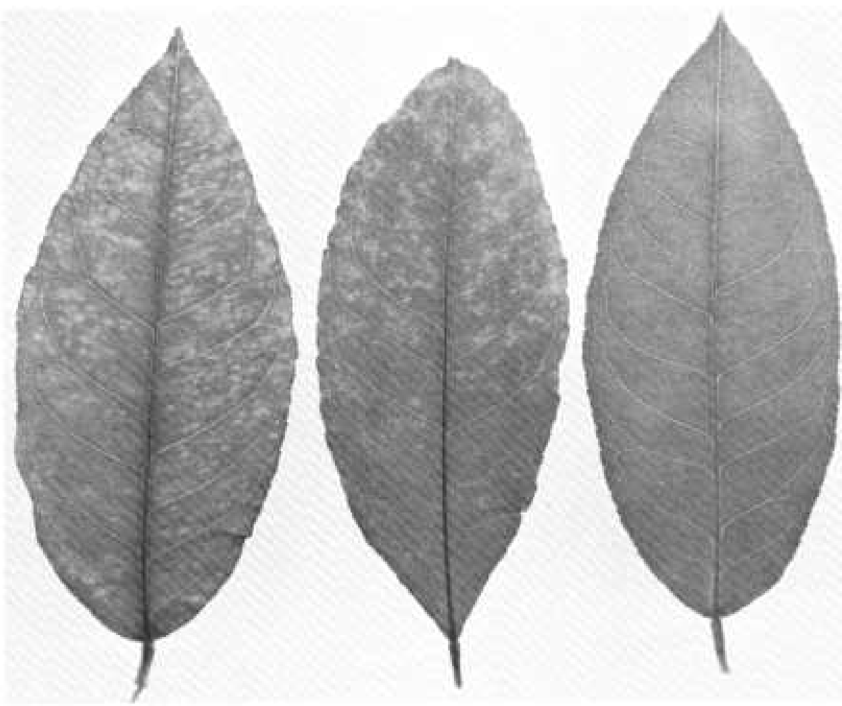

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FIGURE 6.—Psorosis and noninfectious psorosislike symptoms on young leaves of Eureka lemon: *Left,* psorosis; *center,* false psorosis; *right,* normal.

on lemons and could easily be interpreted as a psorosis symptom.

Another condition closely resembling leaf symptoms of psorosis that has been recorded occasionally as psorosis on field trees is shown in figure 7. Extensive studies of this leaf abnormality have been made in California. It is noninfectious and appears to be a genetic abnormality. Seedlings derived from trees showing these leaf patterns also display the same pattern. It has been observed by the author on Cutter and Olinda selections of Valencia orange in California and on Tarocco orange in Sicily.

A third noninfectious leaf abnormality (fig. 8) has been observed in California on lemon trees after severe frost damage. This is a variegation, with some leaves showing chimeral sectors. These symptoms developed on the spring growth of trees on which there had been some freezing back of the small limbs. They were

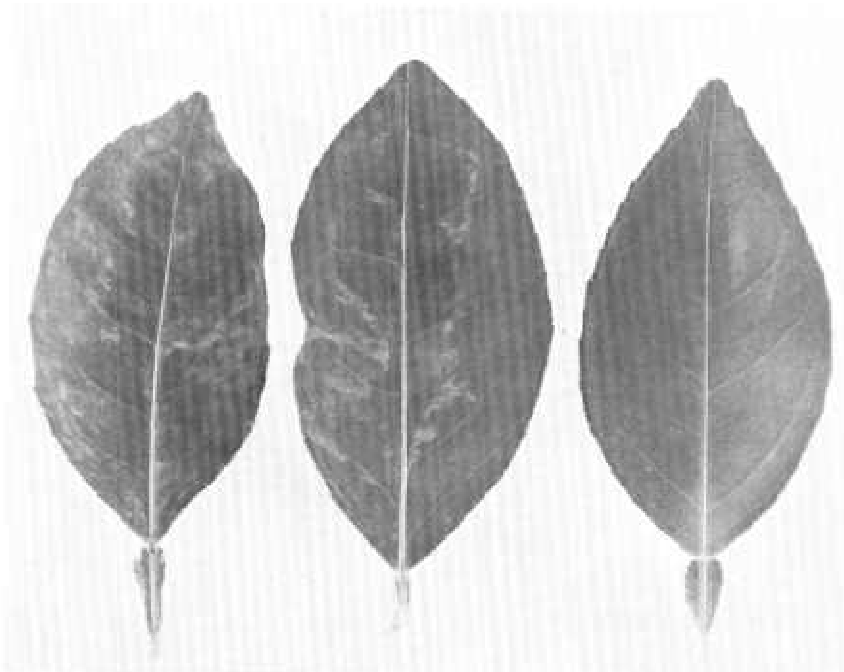

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FIGURE 7.—Psorosislike genetic leaf pattern on sweet orange: *Left,* two leaves show pattern; *right,* normal leaf.

most striking on leaf clusters that developed from buds in close proximity to dead stem tissue. These symptoms were found on virus-free seedlings in nurseries as well as on larger field trees. Individual leaves sometimes were only spotted or stippled and some of them resembled leaves with some effects of psorosis. Leaves of later growth flushes were normal.

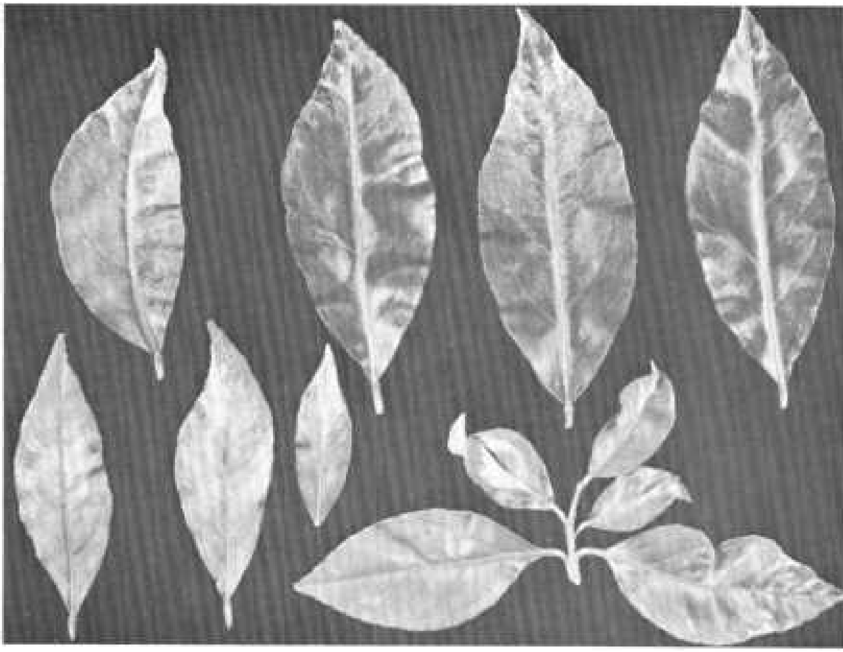

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FIGURE 8.—Variegation and other effects on leaves of lemon shoots that developed near tissues killed by cold.

CACHEXIA (XYLOPOROSIS)

By J. F. L. Childs [1]

Opinions differ regarding the relationship of cachexia (described from Orlando tangelo (*Citrus paradisi* Macf. × *C. reticulata* Blanco)) to xyloporosis (described from Palestine sweet lime (*C. aurantifolia* (Christm.) Swing.)). The available evidence indicates that both diseases are caused by the same virus, and that differences in their respective symptoms are in reality differences in the reaction of different hosts to the same virus (figs. 1 and 2).

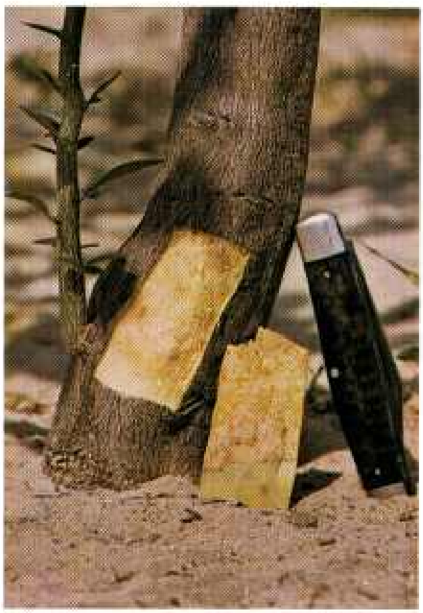

Figure 1.—Orlando tangelo on Rusk citrange rootstock 30 months after budding. Note severe symptoms of cachexia on Orlando tangelo.

L. C. Knorr reported a disease of Murcott orange (probably a tangor (*C. sinensis* (L.) Osbeck × *C. reticulata* Blanco)) in Florida and named it fovea. The symptoms are pitting and pegging

[1] This section was reviewed by E. O. Olson and A. Bental.

FIGURE 2.—Hamlin sweet orange on sweet lime stock 10 years after budding. Note mild symptoms of cachexia on sweet lime stock.

of the cambial surface of the bark and wood and starvation symptoms on the foliage, which are much the same as in cachexia. Gum is not commonly associated with the pits and pegs, a symptom more like xyloporosis. Knorr observed that affected trees usually die 1 to 2 years after the first appearance of symptoms. However, his index tests with Orlando tangelo, sweet lime, and Clementine mandarin (*C. reticulata* Blanco) have been inconclusive.

Cachexia virus occurs widely in commercial varieties of citrus, but causes primary symptoms only on certain mandarin (*C. reticulata* Blanco) varieties, mandarin hybrids, sweet lime, and lime hybrids. Severity of symptoms on susceptible varieties ranges from most severe on Orlando tangelo and Parson's Special mandarin to none on Webber tangelo (*C. paradisi* Macf. × *C. reticulata* Blanco). Other species and varieties of citrus, such as sweet orange (*C. sinensis* (L.) Osbeck), sour orange (*C. aurantium* L.), and grapefruit (*C. paradisi* Macf.), may carry the virus without expressing recognizable symptoms.

SYMPTOMS

The symptoms may be divided roughly into (1) primary damage to tissues by the virus—gum impregnation of the phloem tissues and pitting of the cambial face of the wood and pegging of the bark, followed by necrosis and cankers of the bark that may cause partial girdling and (2) secondary damage—sparse chlorotic foliage, yellow midrib, and retarded growth, which are mainly starvation symptoms resulting from girdling. When tolerant varieties such as sweet orange or grapefruit are grown on susceptible rootstocks, cachexia may cause severe starvation symptoms and stunting of the top through injury to the rootstock.

Indexing Procedure

Several test plants—Rangpur lime (*C. reticulata* var. *austera* hybrid), Palestine sweet lime, and certain tangelo varieties—have been widely used as indicators for cachexia virus infection. None appear more satisfactory than Orlando tangelo (fig. 3). Rangpur

Figure 3.—Marsh grapefruit on Orlando tangelo rootstock with early symptoms of cachexia. (Courtesy of E. C. Calavan.)

lime is also susceptible to exocortis virus. Consequently, the diagnosis of cachexia is not always as clear as it should be when both viruses are present. Palestine and Columbia sweet lime (*C. aurantifolia* (Christm.) Swing.) both react more slowly than Orlando. Wekiwa tangelo (*C. paradisi* Macf. × *C. reticulata* Blanco) gives strong symptoms but grows less vigorously than Orlando, at least in Florida.

Experimental studies indicate that cachexia virus is not seed transmitted. Nevertheless as a precaution, seed for test plants should be obtained from cachexia-free parent trees if possible. Seeds are most easily germinated in vermiculite (expanded mica chips) in flats. Seedlings may be transferred to small pots or cans when 15 cm. high.

The test seedlings can be budded or grafted with tissue from candidate trees when 20 to 30 cm. high. After the bud or graft is established and has made some growth, the seedling top may be cut back, the bud or graft allowed to grow, and the plant transferred to the field for observation. Symptoms appear sooner in the field than in the greenhouse. Plants transferred from pots to the field grow well and require a minimum of care. At least three tests should be made for each candidate tree, although some workers use as many as eight.

In test plants consisting of a top grown from a bud or graft of the candidate tree on Orlando tangelo seedling rootstock, the first symptom will be small spots of gum in the phloem or on the cambial face of the bark at the bud union. Pitting of the Orlando tangelo wood, pegging of the bark, and gum impregnation of the bark develop later. On inoculated seedling trees growing on their own roots but not cut back to force the candidate bud, bark symptoms may appear anywhere on the trunk, although they occur most often near the point of inoculation and below large branches.

The Orlando tangelo grows poorly on its own roots in Brazil, according to Ary A. Salibe. Under such circumstances, he recommends budding Orlando on a vigorous stock such as Rangpur lime that was previously inoculated from the candidate tree. If possible, control plants inoculated with cachexia should be grown simultaneously as a guide to the length of the incubation period and the severity of symptoms under local conditions.

Interpretation of Results

Symptoms as described normally appear in 6 to 36 months in Florida. An occasional plant may require 5 years. If no symptoms have appeared on any of the Orlando test plants in 5 years, the candidate can be considered free of cachexia. Appearance of symptoms may be delayed under certain environmental conditions. At such times inoculated control plants are highly important for comparison in the evaluation of results.

TRISTEZA AND SEEDLING YELLOWS

By J. M. Wallace [1]

Tristeza virus infection of intolerant combinations of top and rootstock can be diagnosed by histological examination with considerable accuracy. However, with tolerant combinations and with mild strains of the virus, it is necessary to index in order to avoid tristeza-infected sources of budwood. The lime test provides a reliable and relatively quick index test and is widely used. However, under certain conditions interpretation of the results may be facilitated by testing with susceptible budded trees. Most combinations of sweet orange (*Citrus sinensis* (L.) Osbeck) on sour orange (*C. aurantium* L.) are susceptible. For greenhouse experimental studies, the author has found that navel or Valencia orange on Eureka lemon (*C. limon* (L.) Burm. f.) is a very susceptible combination.

Indexing for the seedling yellows strain requires citrus species that will give the seedling yellows reaction. West Indian lime (*C. aurantifolia* (Christm.) Swing.) reacts to tristeza virus and to the seedling yellows strain, but does not distinguish between them.

Lime Reaction

Mexican lime (*C. aurantifolia* (Christm.) Swing.), also known as West Indian lime, Key lime, and baladi lime, has been adopted as the best indicator of tristeza virus infection. Leaves of this lime develop distinctive vein clearing when infected, which seems to be specific for the tristeza virus. Other types of lime as well as other kinds of citrus develop similar vein clearing, but none is better than Mexican lime.

Limes infected with tristeza virus also develop stem pitting, which per se cannot be considered specific for tristeza, but substantiates the diagnosis when combined with the leaf symptoms. Infected lime plants may be slightly, moderately, or severely stunted. However, the degree of vein clearing, stem pitting, and stunting on lime varies widely with different strains of tristeza virus. No other citrus virus has been found to produce this set of symptoms on lime.

Seedling Yellows Reaction

Other indicator varieties are necessary to indicate the presence

[1] This section was reviewed by Lilian R. Fraser, H. Schneider, and T. J. Grant.

of the seedling yellows strain. Lemon (*C. limon* (L.) Burm. f.), sour orange (*C. aurantium* L.), and grapefruit (*C. paradisi* Macf.) are good indicators for that purpose. On the basis of extensive studies of strains of seedling yellows virus, the author prefers Eureka lemon. However, the scarcity of seeds in Eureka lemon fruits and the lack of uniformity among the seedlings introduce some problems not found with sour orange and certain varieties of grapefruit. In some regions sour orange and grapefruit prove satisfactory, but in California Eureka lemon is preferred, because a strain of seedling yellows found there causes no symptoms on standard sour orange.

The seedling yellows virus is known to spread naturally in Australia, South Africa, South America, and some other countries, but it has never been recovered from any commercial citrus plantings in California. Most Meyer lemon trees in California were positive for tristeza when tested on lime. Testing on Eureka lemon demonstrated that all that gave the lime reaction also gave the yellows reaction. Several strains of seedling yellows virus are known, and two or more strains may be present in a given tree.

Indexing Procedure

Seeds of the indicator varieties should be collected, treated, and planted as previously described (see pp. 9–10). Transplanting the seedlings when 4 to 6 inches high encourages establishment and rapid growth. The number of seedlings placed in each pot depends on the size of available containers. A 6-inch pot or 1-gallon can will support four lime or two lemon seedlings until completion of the indexing. Mexican lime and Eureka lemon seedlings are not all alike and offtype plants should be eliminated by careful roguing at time of transplanting. Weak seedlings as well as the large-leaved extra vigorous plants should be eliminated. For uniformity, it is often necessary to discard about one-half of the lime seedlings.

Inoculation from the tree being tested can be made with bark or leaf patches, buds, or grafts. On very small seedlings side grafts are most satisfactory. In this method, part of the scion is exposed and can be examined for survival or "take" without unwrapping or cutting into the plant tissue. Side grafts involve the use of more tissue than is the case with buds or leaf and bark patches, and it is believed that they provide more virus for infection. In the author's studies the total percentages of infection were not significantly different with various kinds of inoculum when the "take" was equally good, but symptoms generally appeared sooner on the seedlings infected from grafts.

Test seedlings should be cut back at the time of inoculation to leave two or three buds above the inoculation site for development of shoots. Growth from twig grafts should be suppressed so as not to interfere with the growth of the seedling. Suitable indicators of seedling yellows should be inoculated at the same time if one wishes to know whether the seedling yellows virus is carried by the tree being tested. Four seedlings of lime and four of a seedling yellows indicator normally provide a reliable test. It is well to include at least two noninoculated plants of each indicator variety as controls.

Symptoms

With most sources of tristeza and with the tristeza-seedling yellows complex, the first observable symptoms on infected lime seedlings are chlorosis and cupping of terminal leaves of the shoots that develop after the plants are inoculated and cut back (fig. 1). In the glasshouse these symptoms may be noticeable 3 to

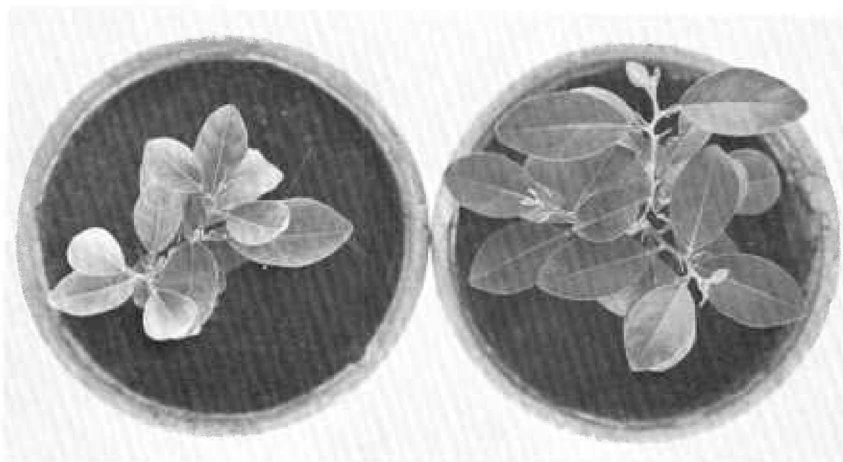

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Figure 1.—Early symptoms of tristeza on Mexican lime: *Left,* stunting, chlorosis, and upward cupping of leaves on infected plant; *right,* normal, noninfected seedling.

4 weeks after inoculation. Young chlorotic leaves may show distinct but slight vein clearing as early as 4 weeks, but usually 5 weeks or longer are required before vein clearing becomes conspicuous (fig. 2, *A*). The amount of vein clearing and its distribution over the plant vary considerably. This is governed to some extent by the strain of virus present, and probably lime seedlings vary somewhat in their response to infection.

Vein-clearing symptoms usually persist long after the leaves mature. Close examination of the undersurface of affected leaves reveals a slight water-soaked appearance of those parts of the veins that appear cleared when viewed by transmitted light (fig. 2, *B*). The author has not found this water-soaking symptom associated with any other citrus virus.

Leaf symptoms nearly always appear within 3 months of the inoculation time, but when all plants of the same group remain negative, they should be observed for a period of 6 months. Test plants should be pruned after 3 months and new shoots forced

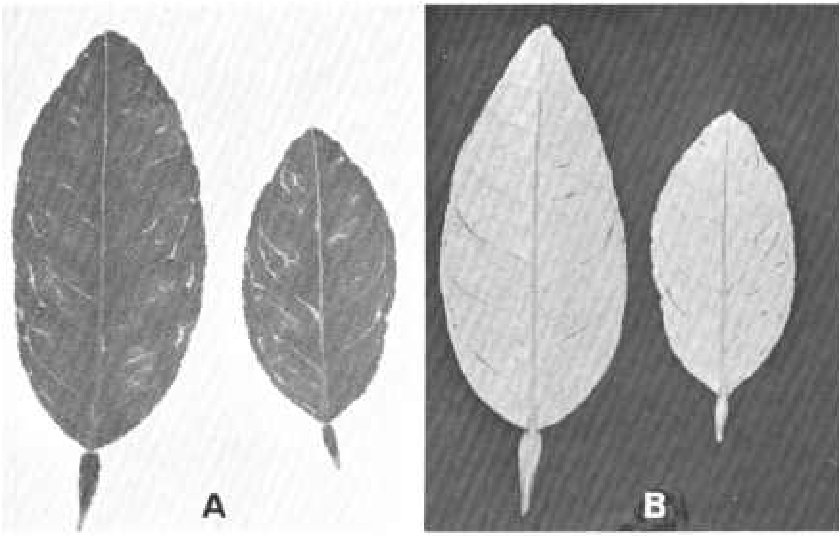

FIGURE 2.—Symptoms of tristeza on Mexican lime leaves: *A*, Vein clearing; *B*, water-soaked appearance of veins on undersurface of affected leaves; affected parts of veins correspond to vein clearings.

from buds near the inoculation site. If no vein clearing appears on this growth within the second 3-month period, the plants should be cut off at that time and examined for stem pitting by stripping the bark from the main trunk and several small branches (fig. 3, *A*). Pitting appears on the base of small, green, hardened shoots at times when it cannot be found on the older stems. At other times pitting can be detected without removal of the bark (fig. 3, *B*). If none of a particular group of lime seedlings show vein clearing or stem pitting within 6 months, it can be concluded that the test is negative, i.e., that the tree under test was free of tristeza virus when the inoculum was taken.

If seedling yellows virus has been introduced into lime test plants, it commonly causes an early and fairly severe reaction, but some strains of tristeza cause symptoms equally as severe. Therefore, lemon seedlings, or another suitable indicator of yellows, must be used to determine whether the inoculum contains seedling yellows virus. With some sources of virus, Eureka lemon seedlings develop symptoms of yellows before lime plants in the same test show any symptoms. If lemon seedlings are infected with seedling yellows virus, growth is retarded and terminal leaves of newly developing shoots are chlorotic and smaller than normal (fig. 4). Later the symptoms of seedling yellows advance rather rapidly. Some strains of the virus cause severe stunting and almost a complete cessation of growth (fig. 5). There is a wide range of severity dependent on the source or strain of virus.

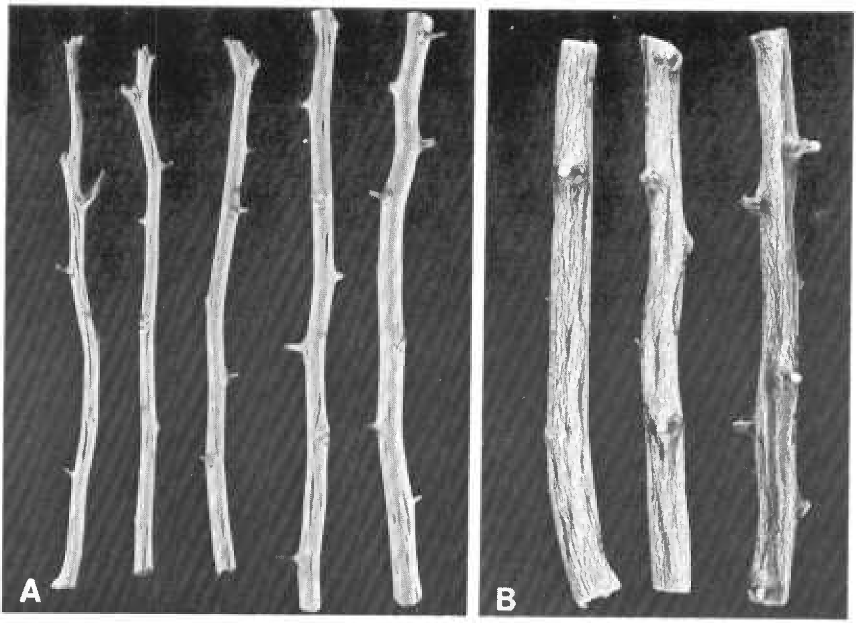

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FIGURE 3.—Tristeza stem pitting on Mexican lime: *A*, Twigs with bark removed showing characteristic wood pitting; *B*, older stems on which pitting can be seen without removing bark.

After showing good yellows symptoms, seedlings of lemon, sour orange, and grapefruit may recover by the renewal of growth with little or no disease effects. However, in California some strains of seedling yellows virus have been found from which lemons failed to recover. The rate of recovery seems to be governed by the virulence of the virus strain. In some instances, the affected plant may show strong symptoms in the terminal part of the stem for a relatively short time and then produce growth that gradually becomes nearly normal. In other cases, several months may elapse before any recovery is initiated, and this may come about slowly by the production of mildly affected or symptomless shoots from below the affected terminal of the plant.

Studies have shown that after plants of lemon, sour orange, and grapefruit recover and no longer display seedling yellows symptoms, the virus retained by them will cause the tristeza lime reaction and decline of tristeza-susceptible budded trees, but will not cause seedling yellows. The disappearance of the yellows virus takes place gradually during plant recovery, and sometimes several months elapse before this component of the virus complex is no longer present.

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FIGURE 4.—Seedling yellows symptoms on Eureka lemon: *A*, Healthy control; *B*, plant 36 days after graft inoculation.

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FIGURE 5.—Seedling yellows-affected Eureka lemon 1 year after inoculation with virulent strain of seedling yellows virus from which lemon seedlings seldom recover.

EFFECT OF OTHER VIRUSES

Mixtures of tristeza and other viruses occur rather commonly. However, a thorough or complete experimental study has not been made of the effect of other viruses on tristeza symptoms. Most of the other viruses seem not to affect the symptoms of tristeza and seedling yellows on indicator seedlings. Two exceptions are psorosis and greening disease viruses.

If psorosis virus is also present, its symptoms usually appear first and may mask or prevent early development of distinct vein clearing on lime, especially if the strain of psorosis is one that causes shock symptoms. However, typical vein clearing will be evident in later flushes of leaves if tristeza virus is present in the inoculum. The presence of psorosis virus seems to have no effect on subsequent development of stem pitting.

The reactions of sour orange, Eureka lemon, and grapefruit seedlings will not differentiate between seedling yellows and greening disease viruses because both produce severe chlorosis on these varieties. However, if a positive reaction for greening disease (a chlorosis similar to that of iron and zinc deficiencies) is obtained on sweet orange, the presence or absence of seedling yellows must be established by transmission of the virus with the brown citrus aphid (*Toxoptera citricida* (Kirkaldy)). Greening disease virus is believed to be transmitted by psyllids and not by aphids.

EXOCORTIS

By E. C. Calavan [1]

Exocortis is primarily a disease of citrus trees grown on rootstocks of trifoliate orange (*Poncirus trifoliata* (L.) Raf.), citrange (*P. trifoliata* (L.) Raf. × *Citrus sinensis* (L.) Osbeck), and Rangpur lime (*C. reticulata* var. *austera* hybrid). Generally bark scaling on the rootstock and stunting of the top are noted. Sometimes scaling of the stock occurs without noticeable stunting of the top and vice versa. Several strains of exocortis virus exist and host reactions to exocortis infection are greatly affected by environment.

Symptoms

Exocortis virus causes vertical cracking and scaling of the bark (fig. 1), yellow blotching of twigs, and considerable stunting of trifoliate orange, certain mandarin limes (*C. aurantifolia* (Christm.) Swing.), and some citranges (*3, 5, 6, 8, 12*). It causes cracking of the bark and stunting in Cuban shaddock (*C. grandis* (L.) Osbeck), some lemons (*C. limon* (L.) Burm. f.), and sweet limes (*C. aurantifolia* (Christm.) Swing.) (*15*).

On certain seedling clones (U.S.D.C.S. 60–13 and Arizona 861) of Etrog citron (*C. medica* L. var. *ethrog* Engl.) exocortis virus induces cracking of the abaxial surface of the midrib, stem and leaf epinasty, yellow blotching of the stem, corky lesions and vertical cracks on the stem, and dwarfing (*1, 2, 7*). The base of the petiole and lower side of principal veins of younger leaves may be darkened abnormally. Mature leaves of some citron shoots may die and drop. The virus has been transmitted from citrus to petunia (*Petunia hybrida* Vilm.) by dodder (*Cuscuta subinclusa* Dur. & Hilg.) (*14*).

Exocortis has caused severe losses in areas where trifoliate orange, citrange, or Rangpur lime was used as a rootstock. Many kinds of citrus, including sweet orange (*C. sinensis* (L.) Osbeck) and mandarin orange (*C. reticulata* Blanco), may be stunted slightly by exocortis infection, but they usually fail to develop specific symptoms of exocortis.

Dissemination of exocortis virus occurs principally through propagation of infected budwood. Natural spread in California orchards appears to have been slight, but considerable natural

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[1] This section was reviewed by Ary A. Salibe, H. C. Burnett, and E. O. Olson.

BN-28379

FIGURE 1.—Exocortis on 12-year-old trifoliate orange rootstock, partially excavated to show lesions below soil level. Top variety is Temple orange (probably hybrid (*C. sinensis* (L.) Osbeck × *C. reticulata* Blanco)).

spread in greenhouses has been noted (*2*).[2] Seed transmission of exocortis virus has been suspected in Brazil (*13*), but has not been found in Australia (*6*) or California.

INDEX TESTS

Exocortis indexing on indicator plants of citron, such as

[2] Natural spread, so called, probably results from mechanical inoculation by contaminated budding knives, according to the results of S. M. Garnsey (J. F. L. Childs).

U.S.D.C.S. 60–13 or Arizona 861, requires only a few months. The citron test (*2*) is the most rapid and sensitive index for exocortis. Citron clones vary tremendously in their reaction to exocortis infection (*1, 7*), and only the most sensitive are useful as indicators. Two widely used and practical methods of inoculation are illustrated (fig. 2).

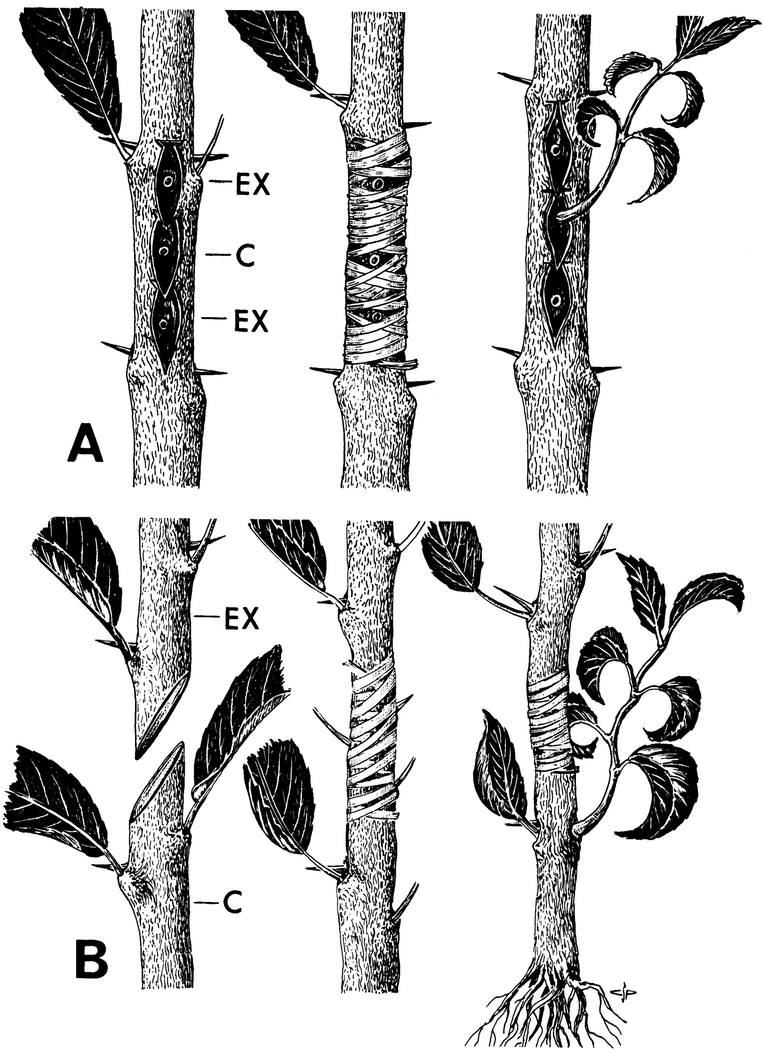

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FIGURE 2.—Two methods of using citron in rapid indexing for exocortis: *A*, Propagation of citron (*C*) on citrus seedling inoculated with exocortis-infected buds (*EX*); *B*, splice grafting of exocortis-infected shoot (*EX*) on citron cutting (*C*), which is then rooted and forced to develop a shoot.

Preferred Method

Two buds from the candidate tree are grafted into each of several seedlings of a suitable exocortis host, such as Rough lemon (*C. limon* (L.) Burm. f.) or Ponderosa lemon (probably a hybrid, *C. limon* (L.) Burm. f. × *C. medica* L.), but are not permitted to develop into shoots. Bark patches may be used. A bud from a healthy plant of a sensitive exocortis-free citron clone, such as C.R.C. 60–13 or Arizona 861,[3] is then inserted in each seedling (fig. 2, *A*). The citron bud may be inserted on the same day as the candidate bud or up to 2 weeks after the candidate bud. Too early infection of a seedling may inhibit growth of the citron bud.

Growth of the citron shoot is encouraged by cutting back or bending the seedlings. The citron scions should be grown as single shoots in a favorable environment in either the greenhouse or the field and pests must be rigorously controlled. Infected plants usually develop symptoms within 1 to 3 months and before the shoots are 2 feet high. Taller symptomless citron shoots may be cut off about 15 cm. above the bud union and a new citron shoot grown for about 3 months.

To avoid overlooking mild strains of exocortis virus, each candidate should be indexed on at least five indicator plants until symptoms appear or until 6 months after inoculation. Noninoculated control plants should be included with the citron index plants to detect possible natural spread of exocortis (*2*). Inspection of citron budwood plants once a week is advisable for the same reason.

The first and most characteristic symptoms of exocortis infection on citron shoots are epinasty of the leaves (fig. 3, *A*) and cracking of the lower side of the midrib (*2*) (fig. 3, *B*). Severely affected leaves curl downward from the tip, and the blades twist to varying degrees from the normal plane. Other symptoms include dwarfing, stem epinasty, yellow blotching of the stem, small corky lesions, and vertical cracking of the stem (fig. 3, *C*). The last symptoms provide corroborative evidence and are especially valuable if one is forced to index on one of the less sensitive strains of Etrog citron.

An alternative method consists of splice grafting a twig from the candidate tree onto exocortis-free citron cuttings (*2, 7*). A 2- to 3-inch section of candidate shoot is splice grafted onto a citron cutting (fig. 2, *B*). The cutting is then rooted and allowed to grow a shoot, but shoots from the candidate graft are removed. This method gives excellent results in the greenhouse and requires no rootstock seedlings.

Other Methods

Rangpur lime seedlings or budded plants have been used extensively as exocortis indicators in Brazil (*9, 11*), California (*2, 3*),

[3] Another citron useful for exocortis indexing is the Beerhalter strain of Mortimer Cohen (Indian River Field Laboratory, Ft. Pierce, Fla., U.S.A.). Most other citron selections are worthless for exocortis indexing. (J. F. L. Childs)

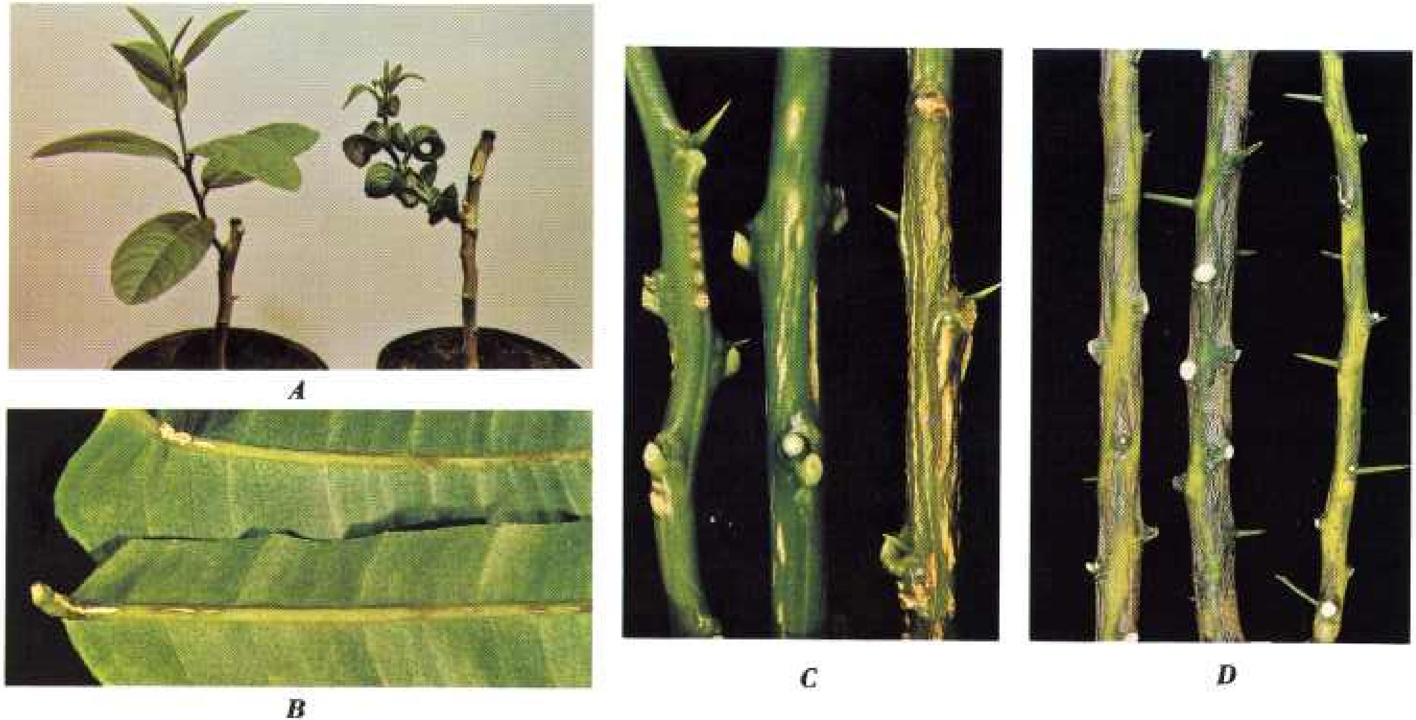

FIGURE 3.—Exocortis symptoms on citron and Rangpur lime: *A*, Epinasty of leaves and stunting in 60–13 citron shoot on inoculated Rough lemon seedling rootstock (right) and healthy control (left); *B*, cracking, scarring, and darkening of underside of midvein on exocortis-infected leaves of 60–13 citron; *C*, corky lesions (left) and cracking in stems (right) of exocortis-infected 60–13 citron; *D*, yellow blotch and slight cracking in stems of exocortis-infected Rangpur lime seedlings.

and Texas (*10*). The principal methods are (1) inoculation of seedlings with buds from the candidate tree and (2) topworking healthy Rangpur lime into one or more branches of the candidate tree. Five or more indicator plants or shoots should be used for each candidate. The use of seedling indicator plants avoids the possibility of accidental inoculation of a candidate tree from grafts used for topworking.

Vigorous Rangpur lime shoots often react to exocortis infection in 4 to 15 months. Symptoms of exocortis in Rangpur lime shoots are yellow bark blotches of variable size and shape (fig. 3, *D*) usually located near thorns, vertical cracks in or near these blotches, limited necrosis, and sometimes scaling around the cracks. Rangpur lime is useful as an indicator in the greenhouse or field. Although Rangpur lime usually reacts to infection with severe strains of exocortis virus, it is less sensitive to infection by milder strains than certain citron clones (*2*).[4]

Long-term indexing of exocortis, by growing a scion of the candidate tree on exocortis-sensitive rootstocks, such as trifoliate orange or Rangpur lime, has been widely used. However, this method is slow and expensive compared with the more rapid citron method. A further disadvantage is that failure of the rootstock to develop symptoms after several years does not insure freedom from exocortis infection (*2*).

The presence of exocortis symptoms in exocortis-sensitive rootstocks of candidate trees or their progeny, determined by direct examination or by the phloroglucinol test (*4*), makes indexing unnecessary.

Incidence of exocortis in susceptible trees can be minimized by propagating from parent trees that are exocortis-negative when indexed on sensitive citron plants.

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[4] In Florida, Rangpur limes, both Florida and Brazilian strains, required 2 to 3 years to develop exocortis symptoms in the greenhouse (J. F. L. Childs).

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STUBBORN

By E. C. Calavan [1]

Symptoms

Stubborn disease affects all commercial varieties of citrus regardless of rootstock. The wide range of symptoms includes bunchy upright growth of twigs and branches, with short internodes and excessive numbers of shoots; multiple buds; thickened and sometimes pinholed bark; slight to severe stunting; small or misshapen leaves or both, which frequently are mottled, dappled, or chlorotic; excessive winter defoliation; unseasonal blossoming; low production of fruit, of which some may be very small, lopsided, or otherwise deformed, including acorn shaped (fig. 1, *B*); dense or cheesy peel; stylar-end greening, or inverted development of ripe coloration (fig. 1, *A*); premature drop of fruit; excessive number of mummified fruits; insipid, sour, or bitter fruit flavor; and large numbers of poorly developed, discolored, and aborted seeds (fig. 1, *C*) (*1*).

Stubborn symptoms are highly variable and frequently only a few are expressed at one time. Trees with severe fruit symptoms of stubborn disease may have relatively normal foliage and shoots; trees with strong foliage and shoot symptoms may bear many normal fruits. Trees may show symptoms on some parts, whereas the remainder of the tree appears normal. Fruit and shoot symptoms tend to be more pronounced on the south half of the tree in hot climates of the northern hemisphere.

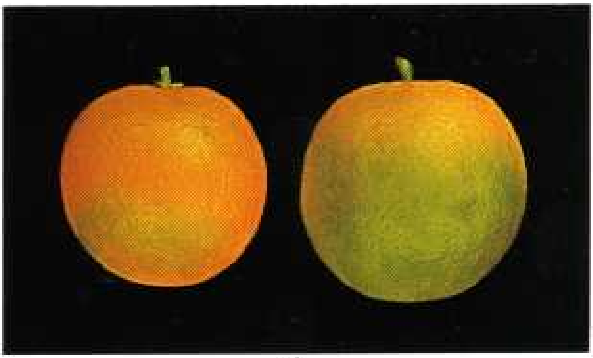
Inoculation Method

Stubborn disease has been repeatedly transmitted to healthy citrus plants by tissue grafts and is believed to be caused by a virus or virus complex. The plants reacting to experimental graft inoculation have ranged from 100 to less than 5 percent. This variation suggests that the pathogen is distributed irregularly in the host. Therefore, considerable replication of candidate tissues and of index plants is needed to obtain reliable data.

A total of 20 or more tissue samples should be put into 10 or more indicator plants. Each tissue sample used as inoculum should be taken from a separate shoot, and shoots should be chosen to represent all parts of the tree as far as possible. Seed for indicator and rootstock plants for index tests should be derived,

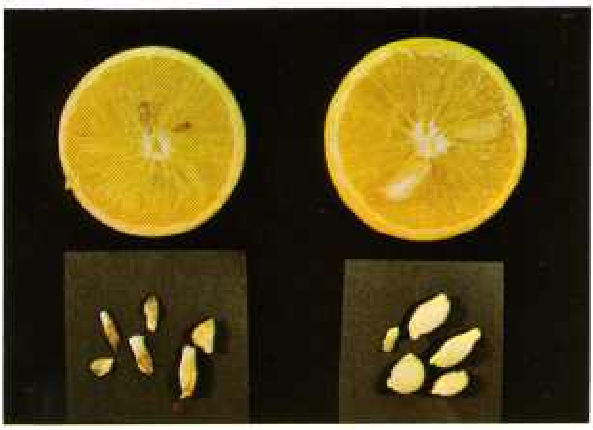
[1] This section was reviewed by J. B. Carpenter and J. Cassin.

when possible, from disease-free parent trees. Indicator plants should be vigorous and free from nutritional problems.



A

B



C

INDICATORS

Seedlings of several varieties of sweet orange (*Citrus sinensis* (L.) Osbeck), tangelo (*C. paradisi* Macf. × *C. reticulata* Blanco), grapefruit (*C. paradisi* Macf.), and mandarin (*C. reticulata* Blanco) are highly sensitive indicator plants for stubborn disease and suitable for indexing purposes. Numerous alternative indicator plants are known, including the clonal seedling lemons previously reported (*2, 3*). Many susceptible varieties may serve as self-indicators, especially in the field.

INDEXING PROCEDURES

The indexing method, described in conjunction with inspection of the candidate tree and its progeny, is at present the best rapid test available for stubborn disease. However, it has not been tested sufficiently to insure complete reliability under all conditions and may fail to detect some cases of stubborn disease.

Short-term indexing requires from 2 to 8 months. Good results have been obtained in heated glasshouses with pencil-sized Madam Vinous or Hinckley sweet orange seedlings inoculated with side grafts or tip grafts from 50 to 100 mm. long (figs. 2 and 3) during the spring or early summer. More than one piece of candidate inoculum (buds or stem pieces) may be grafted into each indicator seedling. Grafts should be protected from drying until they have healed well. Multiple shoot growth should be prevented. Instead, a single unbranched shoot (figs. 2 and 3) should be forced from near the inoculation site and inspected frequently for leaf and shoot symptoms. Several control seedlings should be grafted in exactly the same manner as the index plants, substituting their own or other healthy seedling tissues for candidate inocula.

Stubborn symptoms in glasshouse-grown seedlings of sweet orange include slight to severe stunting, short internodes, and small, cupped, somewhat chlorotic leaves (fig. 4). Marginal and interveinal areas near the tips of nearly grown leaves may be pale green (fig. 5, *A*, *left*). Leaf blades may be variously dappled and mottled with light and dark green (fig. 5, *A*, *center*) or may occasionally show dark-green spotting similar to that observed on stubborn-diseased lemon plants (*2*).

When seedling indicator plants are not available, buds of both the indicator and the candidate plant may be grafted or budded with a third plant that serves as a carrier for both.

Field indexing usually requires from 15 to 24 months after spring or summer inoculation of pencil-sized indicator seedlings with two or more side grafts from 50 to 100 mm. long. Inoculated seedlings usually are grown in the nursery until the following spring, then cut back to about 60 cm. in height, and transplanted to an index plot. Topping or bending plants a month after inoculation forces growth of a single shoot near the inoculation sites and may accelerate the reaction. Excessive growth from the candidate grafts should be prevented, although some foliage is desirable.

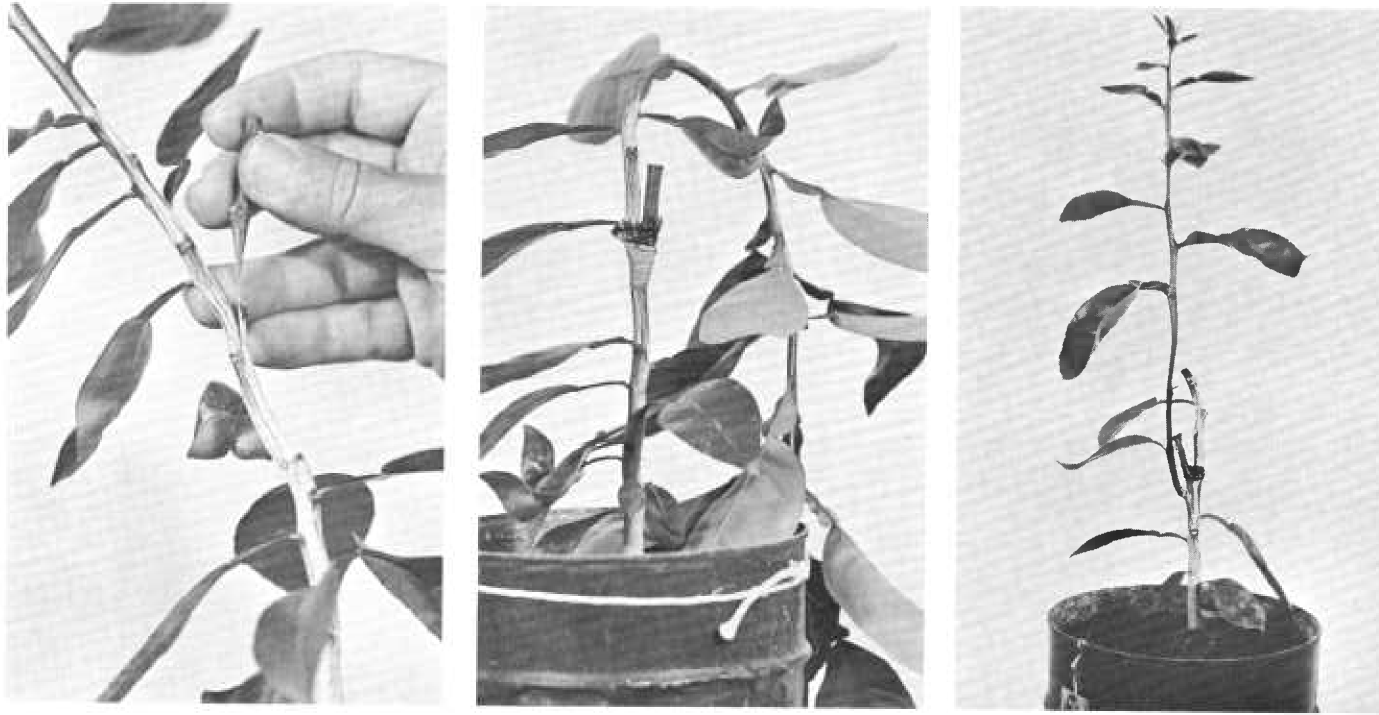


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FIGURE 2.—Side-graft method of indexing for stubborn disease on sweet orange seedling: *Left*, candidate shoot trimmed in V-shape to fit slanting cut in seedling; *center*, side graft, with protective coating of asphalt emulsion on top end and just above tape, is not disturbed when top of seedling is tied down about 3 weeks after inoculation; *right*, single shoot of seedling growing from near side graft 8 weeks after inoculation and 2 weeks after removal of bent-over top.

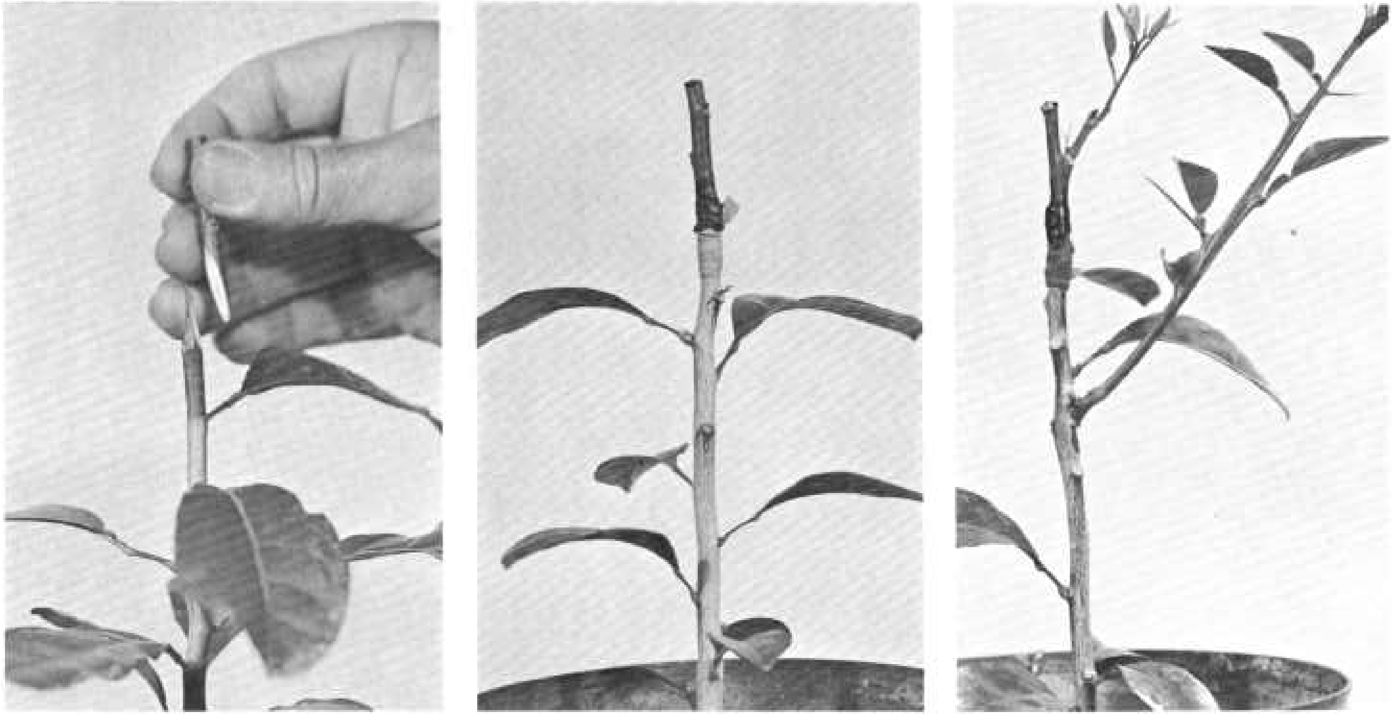

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FIGURE 3.—Tip-graft method of indexing for stubborn disease on sweet orange seedling: *Left*, candidate shoot cut diagonally and matched to beveled stump of seedling; *center*, candidate shoot held in place by tape, and by pin if necessary, and protected by coating of asphalt emulsion at top end and just above tape; *right*, single indicator shoot from seedling is grown—other shoots have been removed and growth from tip graft has been cut back.

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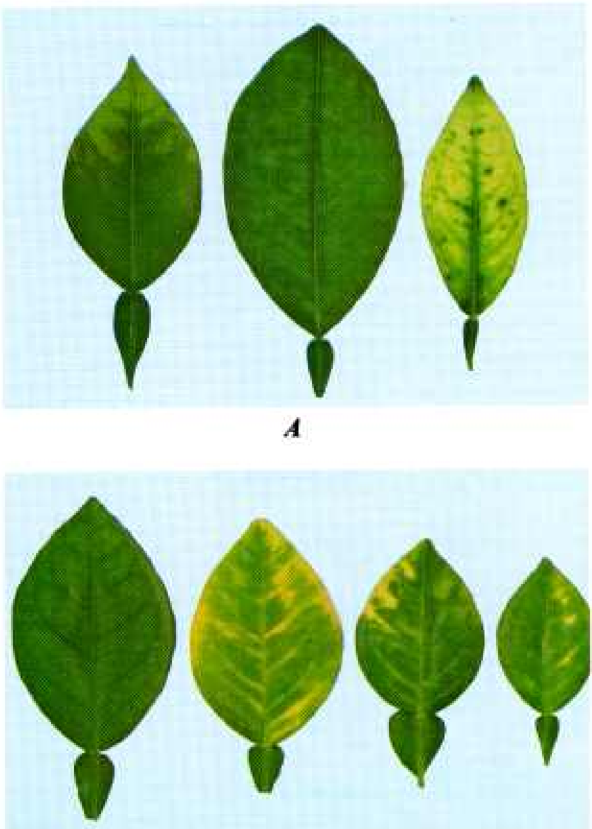
FIGURE 4.—Madam Vinous seedling sweet orange plants: *Left*, healthy control, tip-grafted with healthy tissue. *Right*, plant 3½ months after inoculation in greenhouse with tip graft from stubborn-infected tree; note short internodes and small leaves.



A

B

FIGURE 5.—Leaf symptoms of stubborn disease: *A*, *left*, pale-green marginal and interveinal areas near tip of expanding leaf of Madam Vinous sweet orange seedling in glasshouse; *A*, *center*, light-colored dappling and mottling in fully expanded leaf of Madam Vinous sweet orange seedling in glasshouse; *A*, *right*, dark-green spotting in mature leaf of field-grown Koethen sweet orange seedling; *B*, *left*, normal leaf from control plant; *B*, *right*, field-grown Sexton tangelo leaves showing chlorosis accompanied by yellow streaking along veins.

Positive reactions to stubborn disease infection may develop as follows: General stunting with short internodes, numerous growing points, and small, rigid, often upright, cupped leaves (fig. 6). The leaves may display a variety of pale-green or yellowish patterns in the form of dappling, mottling, interveinal chlorosis, or yellow banding and streaking along the veins (fig. 5, *B*). Some leaves show green spotting on a pale-green to greenish-yellow or creamy-yellow background (fig. 5, *A*, *right*). Leaf symptoms change rapidly. They often resemble patterns caused by nutritional imbalance and are not diagnostic unless absent in the control plants. On severely affected plants many leaves age prematurely and drop during the winter.

Sexton tangelo, Marsh grapefruit, Parson's Special mandarin, and Koethen sweet orange seedlings are among the varieties that have been successfully used in California as indicator plants in the field. Stubborn disease often can be detected by examining the fruit and the vegetative characteristics of a candidate tree and its graft-propagated progeny. Symptoms that are masked in large parent trees often appear well defined in progeny trees in the nursery or young orchard. Annual inspections and accurate records of bud-progeny trees are essential in indexing for stubborn disease.

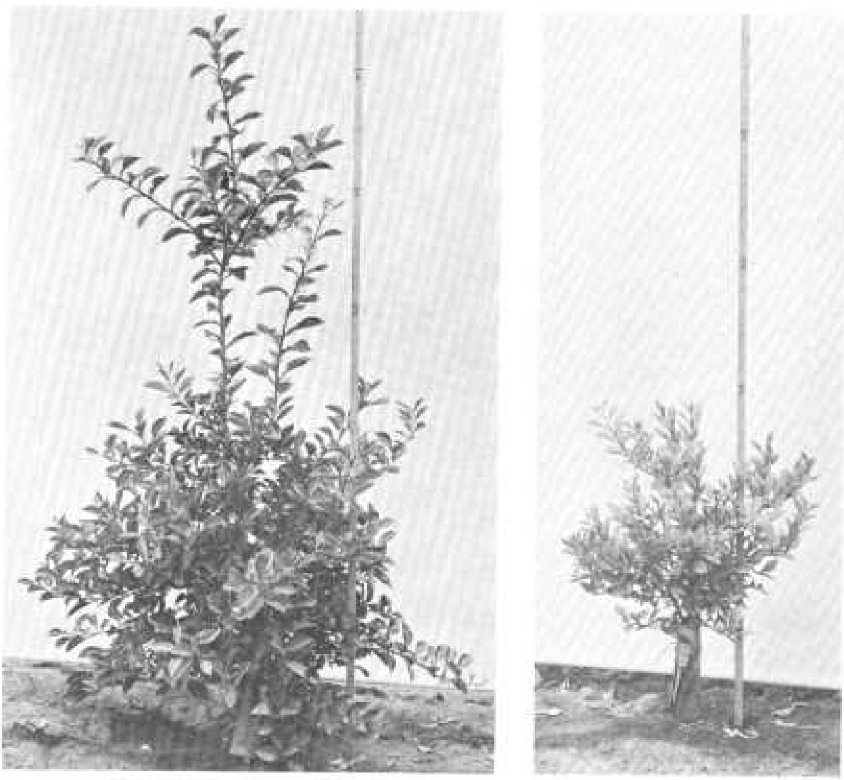


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FIGURE 6.—Sexton tangelo seedlings in long-term index block: *Left*, healthy control; *right*, tree 2 years after inoculation is stunted, with small, upright, chlorotic leaves.

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VEIN ENATION AND WOODY GALL

By J. M. WALLACE [1]

Studies in California have now established that vein enation and woody gall of citrus are caused by the same virus. This virus has been reported from the United States (Calif.), Republic of South Africa, Australia, Peru, and Japan. Numerous kinds of citrus are symptomless hosts of this virus; others develop only the vein enation symptoms. No injurious effects are apparent in conjunction with the latter.

The woody gall phase of this disease is potentially damaging to citrus trees. For example, woody gall has caused some alarm in Peru, where many new plantings of citrus consist of sweet orange (*Citrus sinensis* (L.) Osbeck) on Rough lemon (*C. limon* (L.) Burm. f.) rootstock. These plantings include trees up to 10 years of age, and a high percentage of the trees in some locations have developed extensive galls on the Rough lemon rootstock.[2] No significant reduction in yields of affected trees is apparent, but the trunks of trees with considerable gall development are usually somewhat smaller than normal. This symptom at least suggests that eventually affected trees will not perform as well as those without galls.

The virus is transmitted by at least three species of aphids—*Toxoptera citricida* (Kirkaldy), *Myzus persicae* (Sulzer), and *Aphis gossypii* Glover. Thus, once the virus is introduced to a citrus-growing region, it may become widely distributed within a short time. Infections observed in nursery plantings in Peru are probably caused by aphid vectors as well as from using infected budwood. Some of the nursery trees were stunted by the development of galls when ready for transplanting.

SYMPTOMS AND HOST RANGE

Vein Enation

Symptoms on leaves range from small swellings to conspicuous projections on veins on the under surface of the leaves (fig. 1, *A*). On the upper surface shallow indentations coincide with the peglike structures on the opposite side (fig. 1, *B*). To the touch and the unaided eye, these structures seem sharply pointed,

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[1] This section was reviewed by Lilian R. Fraser and A. P. D. McClean.

[2] Vein enation virus is widespread in South Africa, although the incidence of leaf symptoms is low on field trees and the incidence of galls is even lower (A. P. D. McClean).

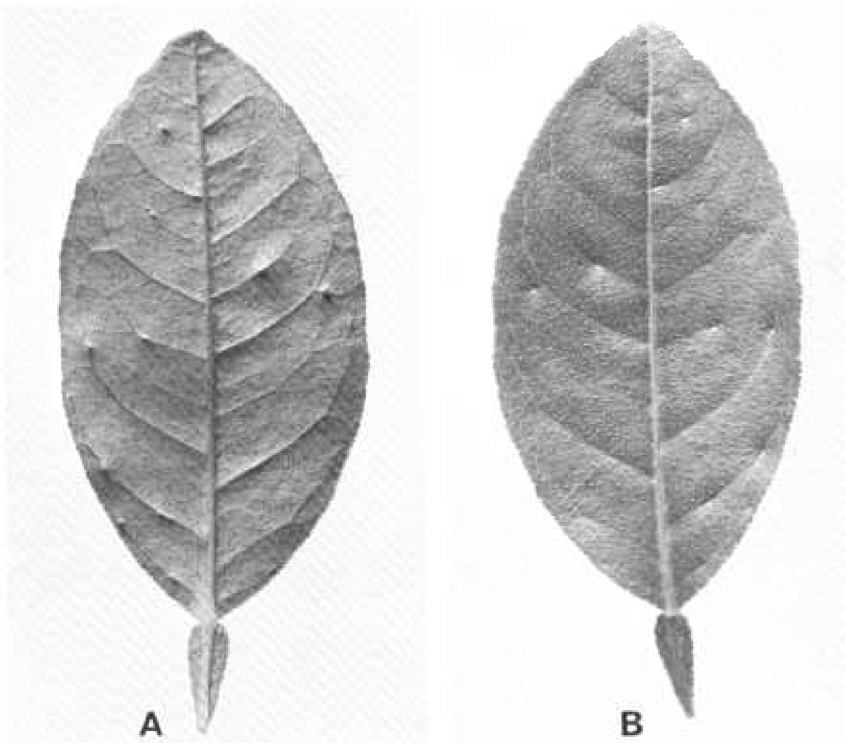

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FIGURE 1.—Vein enations on leaves of Mexican lime: *A*, Vein swelling and enations on under surface of leaf; *B*, indentations on upper surface of leaf corresponding to enations on opposite side.

but on close examination some are found to be knoblike or cup shaped. The number and size of the enations vary widely depending on the variety of citrus affected.

Extensive host-range studies have not been made, but present information indicates that all the commercially used citrus scion and rootstock varieties are hosts of the virus, although some develop no vein enations. Mexican lime (*C. aurantifolia* (Christm.) Swing.), some sour lime varieties, and several sour orange (*C. aurantium* L.) selections develop pronounced enations in California in both the field and greenhouse. Rough lemon and sweet orange develop some leaf enations in the greenhouse, but show less conspicuous symptoms in the field.

Woody Gall

On small green twigs, the galls first appear as slight swellings, over which the bark becomes grayish as the galls enlarge. On seedlings of Mexican lime and Rough lemon, galls may be recognized on small green twigs 8 weeks after infection, but they are inconspicuous in the early stages. Usually galls on the main stem of seedlings develop close to thorns (fig. 2, *A*) and near the graft-inoculation sites.

As their size increases, galls become irregular in shape and the bark covering the gall tissue becomes rougher and lighter colored than normal. Adjacent galls may coalesce as they become larger and form "cauliflowerlike structures" (fig. 2, *B* and *C*), as originally described by Lilian Fraser. The gall tissue is very hard and extends in a cone shape from the point of origin, which is usually in or near the pith region of the stem (fig. 2, *D*).

Characteristically the cambial face of the gall is lightly pitted because of numerous undeveloped or latent buds and is light green because of chlorophyl (fig. 3, *A*). Shoots develop sometimes from the latent buds on old galls. These characteristics assist in distinguishing true woody galls from other swellings near the base of thorns and near leaf or twig scars (fig. 3, *B*) that superficially resemble the early stages of woody gall. When the bark is removed from such structures, they are found to consist of a raised portion of wood with a short, sharply pointed projection folded over a slight crease in the wood (fig. 3, *C*). These structures apparently are undeveloped thorns.

When grown in soil, the roots of infected lime seedlings occasionally develop a few galls, but when maintained in water culture, great numbers of galls develop over the root system (fig. 3, *D*).

Information is limited on the kinds of citrus that develop woody galls. The galls appear commonly on Rough lemon and Mexican lime. In California studies galls failed to develop on sweet and sour orange, Eureka lemon, *C. excelsa* Wester,[3] and Palestine sweet lime (*C. aurantifolia* (Christm.) Swing.) even when infected plants were experimentally wounded. One seedling of Rangpur lime (*C. limonia* Osbeck)[4] developed a few galls, but others did not, even after experimental wounding. However, an unidentified mandarin-lime variety, recorded only as Indian lime, is very susceptible to gall development.

Indexing

Mexican lime and sour orange are good indicators. Test seedlings should be inoculated with a twig graft and examined later for leaf enations. Vein swellings or the early stages of enations or both sometimes appear 4 to 5 weeks after inoculation by grafting.

Other Kinds of Galls on Citrus

Several types of galls or gall-like growths not associated with

[3] According to P. C. Reece, this variety is known by the common names Le Nestour and Limon Real (J. F. L. Childs).

[4] The author prefers *C. limonia* Osbeck over *C. reticulata* var. *austera* hybrid for Rangpur lime. The taxonomy and nomenclature of this variety of citrus are discussed by P. C. Reece in the second edition of The Citrus Industry. (J. F. L. Childs)

the vein enation virus have been observed on citrus. Some appear to be genetic, or at least not of virus origin, since they cannot be reproduced. Several transplants of tissue from these galls resulted in enlargement of the transplanted tissue only. Inasmuch

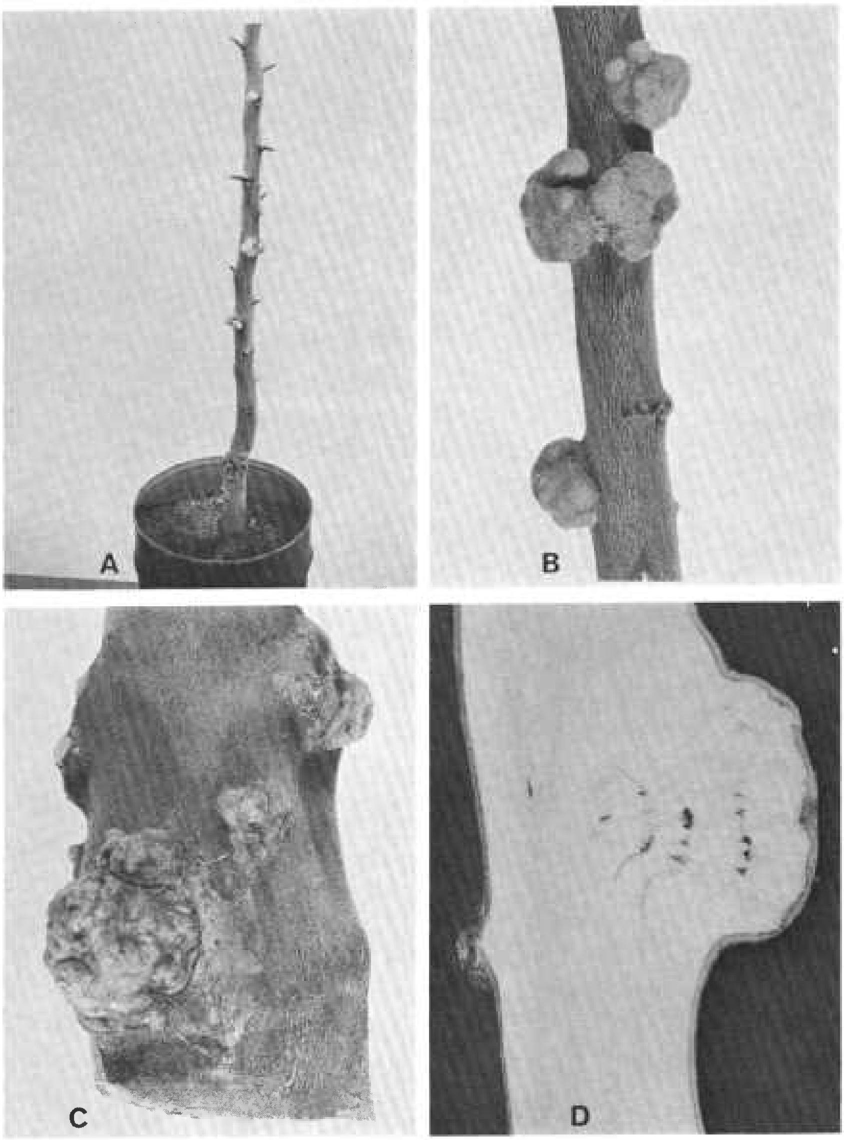

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FIGURE 2.—Woody galls on Rough lemon: *A*, Galls developing near thorns; *B*, galls becoming irregular in shape and coalescing; *C*, cauliflower-shaped gall on orchard tree; *D*, section through small gall showing V-shaped development from point near center of stem.

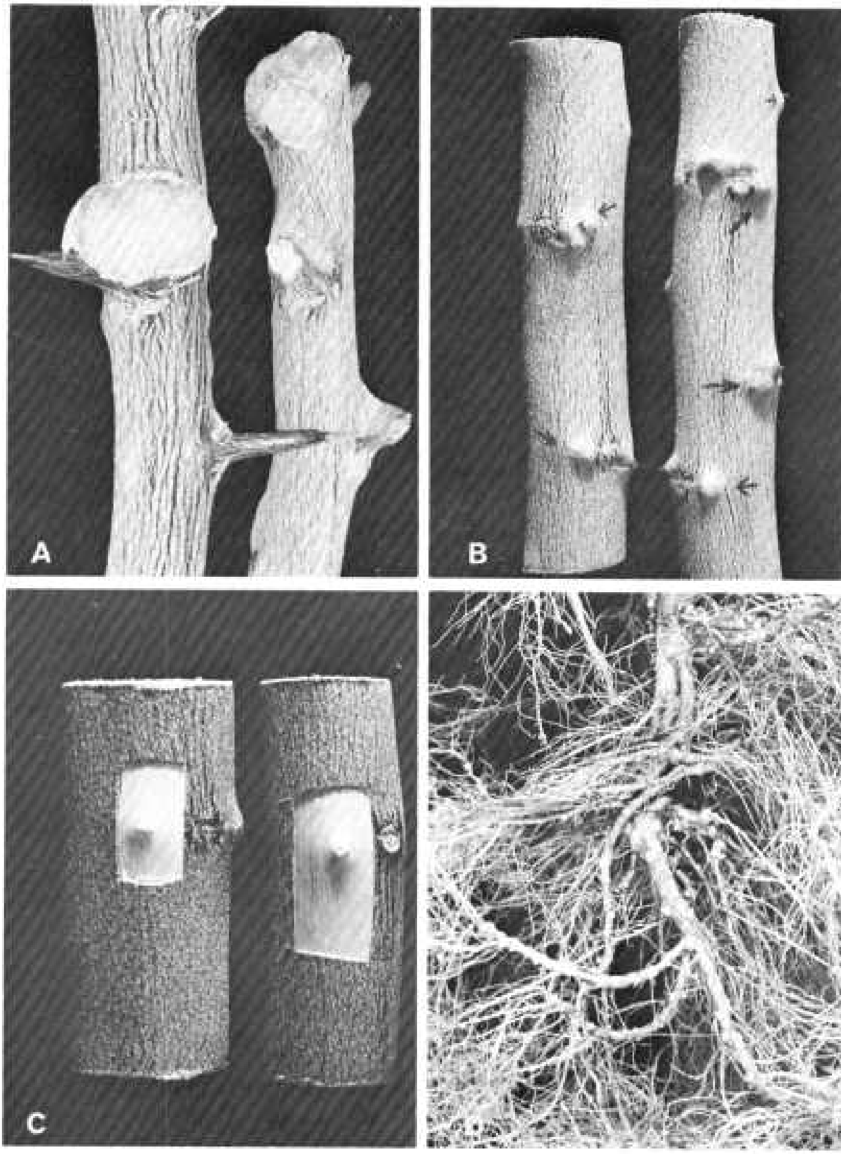

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FIGURE 3.—Woody gall of citrus: *A*, Virus-induced woody gall with bark removed to show pitted appearance (adventitious buds); *B*, swellings near thorns of Rough lemon resembling early stage of woody gall; *C*, bark removed from swellings shown in *B* revealing short thornlike structures; *D*, woody gall development on roots of Mexican lime grown in water culture.

as some of them may closely resemble true woody galls, diagnosis is best made by inoculation from affected trees to indicators for vein enation virus.

GUMMY BARK OF SWEET ORANGE

By Farid Nour-Eldin [1]

Gummy bark disease of sweet orange (*Citrus sinensis* (L.) Osbeck) was first reported (*5*) to affect baladi [2] sweet orange trees grafted on sour orange (*C. aurantium* L.) rootstock in Egypt. Later it was found (*3*) to affect several other sweet orange varieties: Valencia, navel, Sukkary, Khalili white, Egyptian blood, and Sanguinoval. More than 50 percent of the examined trees exhibited symptoms of this disease. Gummy bark was observed on sweet orange trees in Saudi Arabia (Nour-Eldin, 1959, unpublished) and in the Sudan (Childs, 1964, unpublished). A mild form of gummy bark was reported from Florida (*4*) and Egypt (*6*) on sweet orange trees grafted on Rough lemon (*C. jambhiri* Lush.) (see p. 92) rootstock.

Symptoms

Orchard trees affected with gummy bark disease are usually stunted. When the bark of affected trees is scraped carefully, circumferential streaks of reddish-brown, gum-impregnated tissue can be easily seen, particularly near the bud union (fig.1). Occasionally the discoloration extends 24 inches or more into sweet orange tissue above the bud union. In such severe cases, streaks of gum-impregnated tissue may be either longitudinal or circumferential and dark brownish. Bark discoloration has never been observed on the sour orange rootstock of infected trees.

In addition to gum impregnation of the bark, pitting of the wood of sweet orange is common. Two types have been observed. One gives a grooved or channeled appearance to the cambial face of the wood, and the other is a round conoid type of pitting (fig. 2). The latter is the more common. Gum impregnates the inner tissue of the pegs, projecting from the cambial face of the bark and coinciding with the pits in the wood. Severe stem pitting does not necessarily accompany gum impregnation of the bark. In many cases stem pitting is mild and superficial. When severe pitting occurs on the scion, the sour orange stock occasionally exhibits very slight wood pitting.

Transmission Tests

Gummy bark disease was transmitted experimentally by bud in-

[1] This section was reviewed by L. C. Knorr and J. F. L. Childs.
[2] Arabic word meaning local.

oculations from infected trees to healthy sweet orange trees budded on sour orange rootstock (Nour-Eldin, unpublished). About 5 years after inoculation, a red-brownish circumferential line was observed at the bud union after scraping away the corky surface of the bark. Dark-brownish streaks can be seen in various degrees of severity after scraping the bark on older affected trees. Wood pitting may be mild on 5-year-old trees, but is seldom clearly apparent until a year or two later.

Indexing of infected sweet orange trees was performed on rootstocks of Orlando tangelo (*C. paradisi* Macf. × *C. reticulata* Blanco), sour orange, Rough lemon, *Poncirus trifoliata* (L.) Raf., citrange (*P. trifoliata* (L.) Raf. × *C. sinensis* (L.) Osbeck), citron (*C. medica* L.), and Rangpur lime (*C. reticulata* var. *austera* hybrid). Results showed that gummy bark disease usually is accompanied by several other viruses, e.g., psorosis, cachexia, exo-

FIGURE 1.—Sweet orange on sour orange rootstock, showing gum impregnation of bark and pitting of wood above union.

cortis (Rangpur lime disease), and bud union constriction of sweet orange trees on Rough lemon rootstock. Consequently, a test plant highly specific for gummy bark virus is required.

In one of these tests, an indexed tree induced gummy bark symptoms on sweet orange grafted on sour orange and bud union constriction (fig. 2) of sweet orange grafted on Rough lemon rootstock. When grafts from the same source were used to inoculate healthy sweet orange trees on Orlando tangelo rootstock, the rootstock did not react to give symptoms of cachexia disease, indicating that gummy bark disease is not caused by the cachexia virus (*1*, *2*). This particular indexed tree was found to be carrying exocortis virus together with gummy bark disease. Nevertheless, exocortis was not found in other indexed trees that induced gummy bark symptoms on sweet orange trees grafted on sour orange rootstock and bud union constriction of Rough lemon rootstock. This finding indicates that exocortis (Rangpur lime disease) does not induce the gummy bark symptoms described on sweet orange trees grafted on sour orange or Rough lemon rootstocks.

FIGURE 2.—Sweet orange on Rough lemon rootstock, showing constriction and pitting at union.

Previous indexing of virus-infected trees showed that Rough lemon rootstock always developed bud union constriction when gummy bark symptoms developed on sweet orange trees grafted on sour orange rootstock. This suggests that gummy bark of sweet orange on sour orange stock and bud union constriction of sweet orange on Rough lemon stock are caused by the same agent.

Comparison of infected trees with healthy ones shows that the former are much smaller and thinner as a result of the reduced growth and severe leaf drop that occur during the winter. Setting of fruit is affected as trees become old enough to bear fruit.

INDEXING PROCEDURE

Identification of this disease is based on the development of gum deposits in the bark of sweet orange trees grafted on sour orange rootstock. Another scion-stock combination that reacts to this disease is sweet orange on Rough lemon rootstock. Symptoms, as described above, develop on inoculated sweet orange trees grafted on sour orange in about 5 years.

The symptoms described for infected sweet orange trees grafted on Rough lemon rootstock are clearly apparent 4 years after indexing. When bark is removed from across the bud union, an irregular and almost continuous ring of projections is present on the cambial face of the bark at the bud union line, which fits into the crease in the wood (fig. 2). Gum deposits are present in the bark tissue of these projections. Mild stem pitting and bark discoloration can be observed on both scion and rootstock. Such conspicuous symptoms make this combination of sweet orange on Rough lemon rootstock very useful as an indicator plant for gummy bark disease of sweet orange on sour orange rootstock.

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YELLOW VEIN

By L. G. Weathers [1]

Symptoms

Symptoms of yellow vein are similar in different citrus species and varieties, but are most striking in West Indian lime (*Citrus aurantifolia* (Christm.) Swing.), Rough lemon (*C. limon* (L.) Burm. f.), and Etrog citron (*C. medica* L. var. *ethrog* Engl.). The first symptom to appear on the developing leaves is yellowing of the main veins. Yellowing is usually continuous along the petiole, midrib, and main veins (fig. 1). Smaller veins may not be uniformly affected, and frequently there are yellow areas separated by areas of normal green. Chlorosis is sometimes more pronounced on one half of the leaf than on the other, and it appears on both the upper and lower surfaces of the leaf.

[1] This section was reviewed by E. C. Calavan and J. B. Carpenter.

Figure 1.—Etrog citron leaves with yellow vein virus alone (left) and with yellow vein and vein enation viruses (right).

In some cases inoculated plants fail to show typical yellow vein symptoms and in many cases plants with mild symptoms recover; i.e., no further symptoms develop after the initial appearance of symptoms. Yellow vein symptoms are almost completely masked in plants infected with both yellow vein and psorosis viruses. However, in the presence of vein enation virus, symptoms of yellow vein are greatly enhanced and are more severe than in plants infected with yellow vein virus alone or in plants containing both yellow vein and psorosis viruses.

All available evidence indicates that yellow vein virus is not widely distributed and may occur naturally only in the few limequat trees in which it was originally described in California.

Indexing Procedure

The unusual and erratic behavior of yellow vein virus when it occurs alone and in combination with psorosis makes identification of yellow vein virus unreliable under those conditions. Yellow vein virus is diagnosed more reliably by combining it with vein enation virus. The recommended procedure is as follows: Inoculate young West Indian lime, Rough lemon, or citron plants with a pure culture of vein enation virus. Any time after the plants show well-developed enation symptoms, graft inoculate these plants with tissue from plants suspected of having yellow vein virus. If the yellow vein virus is present, severe yellow vein symptoms will appear in the developing leaves of the doubly infected plants after 2 to 6 weeks.

SATSUMA DWARF

By Shoichi Tanaka [1]

Dwarf disease of satsuma orange (*Citrus reticulata* Blanco) has been recognized for more than 30 years in Japan. At the present time the disease is known throughout Japan and is of economic importance in several prefectures. Spread occurs mainly through propagation of infected budwood. Affected trees deteriorate slowly and growers usually postpone removing them. This contributes to inferior fruit production.

Symptoms

From May to June, when leaves begin to elongate, is the best time to detect symptoms of satsuma dwarf. During this period very young leaves of even mildly affected trees curl downward (abaxially). However, as the leaves mature, symptoms become difficult to detect.

On severely affected trees, leaves remain abnormal even after elongation and hardening. They show abnormal shapes of two types—narrow boat shape and dwarfed spoon shape. Only one type may appear on a single twig, but generally both appear together. Also, internodes are usually shortened, giving the twig a witches'-broom appearance.

Fruits on mildly affected trees are normal or nearly so, but when leaf symptoms are severe, the fruit is usually smaller and of inferior quality, with the rind thickened at the stem end of the fruit.

Symptoms occur mainly on spring growth. Summer and autumn flushes show no symptoms even though the trees are severely diseased. When diseased scions are grafted on a healthy tree in the spring, all the buds that come out and develop later that year appear healthy. However, all the buds that come out during the following spring from summer scions show abnormally shaped leaves. When infected scions are top-grafted on 15-year-old satsuma trees, symptoms soon appear on other parts of the tree and entire branches show symptoms several years after inoculation. When this experiment is repeated with young nursery trees, however, all of them show symptoms by the following spring.

Mechanical Transmission

A virus has been transmitted mechanically from satsuma dwarf-

[1] This section was reviewed by Shunichi Yamada and J. M. Wallace.

infected citrus trees to herbaceous plants. Blackeye cowpea (*Vigna sinensis* (Torner) Sari), Satisfaction kidney bean (*Phaseolus vulgaris* L.), and white sesame (*Sesamium indicum* L.) gave the strongest reactions of 35 species of legume and many nonleguminous species tested. Cross-inoculations of the virus from satsuma dwarf-affected trees, between sesame and Blackeye cowpea or Satisfaction kidney bean, were positive. Therefore, the virus is believed to be identical with the so-called satsuma dwarf virus. Sesame proved to be susceptible only to satsuma dwarf virus, but nonsusceptible to tristeza and vein enation viruses, which are commonly carried in satsuma orange trees.

Sesame is more heat tolerant than most leguminous plants and is useful as an indicator for this virus even in the summer. When sesame plants were exposed to a temperature above 34° C. just after inoculation, they usually showed no symptoms, but sometimes systemic infection appeared 10 days after inoculation. On the other hand, when inoculated sesame plants were kept more than 8 hours at 25°, they showed striking symptoms even if exposed subsequently to temperatures higher than 36°. To date the virus has not been transmitted from legumes or sesame to satsuma orange. When Mexican lime seedlings were inoculated from satsuma dwarf-affected trees as well as apparently healthy trees, tristeza symptoms invariably developed.

Procedure for Mechanical Transmission

To perform the herbaceous plant test for satsuma dwarf, the following procedure is recommended: Indicator plants—white sesame, Blackeye cowpea, and Satisfaction kidney bean—should be grown in small pots or cans in the greenhouse. The white sesame seedlings are inoculated with expressed sap on the first or second true leaves. Seedlings of cowpea or kidney bean are inoculated on pairs of the primary leaves.

The sap should be prepared from young shoots or fruit rind from satsuma dwarf-affected trees by grinding with an equal to twice equal volume of 0.05 to 0.10 molar solution of potassium acid phosphate or phosphate buffer of pH 7.0 in a small mortar. The plants are inoculated by the usual rubbing method, using carborundum as an abrasive. Inoculated plants should be kept in the screenhouse at a temperature below 32° C. The optimum temperature is about 28° for sesame and about 25° for the legumes.

Diagnosis

The standard and most reliable method of diagnosis is with white sesame. Several days after inoculation the plants develop local lesions, which are chlorotic spots at first and later become necrotic spots with a yellowish halo. The systematically infected leaves first exhibit vein clearing, malformation, and curling. In later stages these leaves show vein necrosis. Necrosis spreads to the adjacent mesophyll tissue causing the death of the entire leaf. (Figs. 1 and 2.)

A

B

C

FIGURE 1.—Symptoms of virus from satsuma dwarf-affected trees on white sesame: *A*, Local lesions and vein clearing; *B*, vein clearing, yellowing, and dwarfing on systemically infected leaves; *C*, yellowing and necrosis in later stage.

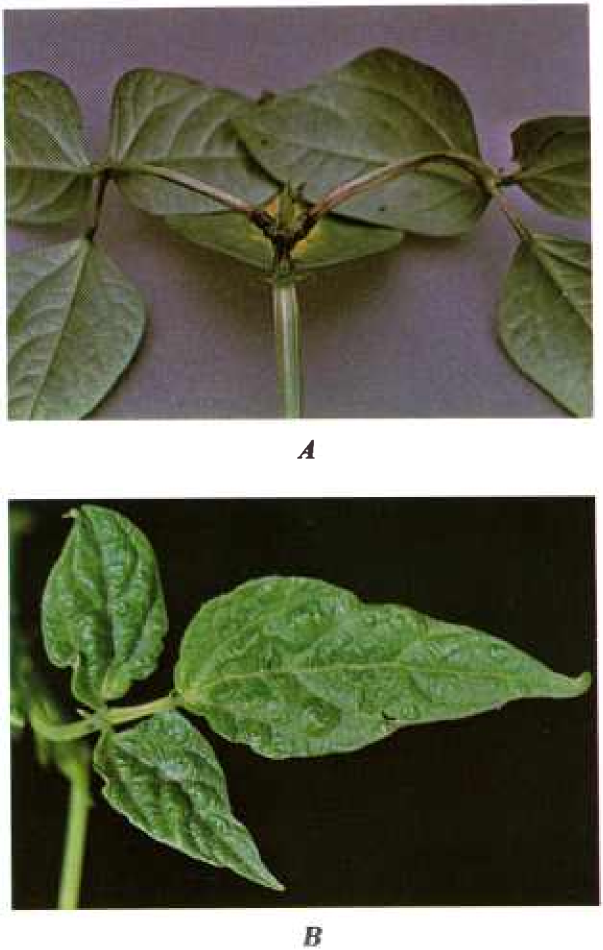

FIGURE 2.—Symptoms of virus from satsuma dwarf-affected trees on Satisfaction kidney bean: *A*, Necrotic streaks on petioles and stem; *B*, vein clearing and mosaic.

IMPIETRATURA

By GAETANO RUGGIERI [1]

Impietratura or stonelike disease of citrus fruits has been known for many years in Sicily and more recently in several other countries of the Mediterranean basin. It affects chiefly orange (*Citrus sinensis* (L.) Osbeck), but it has been found in grapefruit (*C. paradisi* Macf.), Clementine mandarin (*C. reticulata* Blanco), and tangelo (*C. paradisi* Macf. × *C. reticulata* Blanco) trees. It has not been found in lemon (*C. limon* (L.) Burm. f.).

SYMPTOMS

The disease can be detected, especially when fruits are green and actively growing, by noting the presence of small fruits of one-fourth to one-third their normal size near normal-sized fruits on the same tree. The percentage of small fruits varies considerably, but in extreme cases trees produce only small fruits.

Three kinds of fruits may be picked from an affected tree: *Normal* ones of standard shape and size, with an elastic rind and without gum pockets in the albedo; *apparently normal* fruits of standard shape and size, with an elastic rind except over gum pockets in the albedo, and no gum pockets under the calyx; and *very small* fruits of one-half to one-third normal size, pear shaped, and very hard, with gum pockets in the albedo around the fruit and also under the calyx. Less often the vascular bundles of the central axis are invaded with gum. Small fruits always show severe internal characters of the disease; normal-sized fruits have milder symptoms or none at all. Hard, brown, gum-beaded areas in the albedo may be seen by cutting across the fruit near the base and in the part under the stem (fig. 1). Gum may be present in the vascular bundles of the stem as much as several centimeters away from the fruit.

In the most severe cases, the gum-containing tissues may advance inward toward the pulp and thus affect the central axis (core) and the peripheral juice vesicles, which become dry and

[1] This section was reviewed by Henri Chapot. Shortly before the author's death, he prepared a brief statement (Feb. 23, 1965) on the method he used to transmit impietratura. The symptoms described here were taken from Chapot, Henri, Impietratura in Mediterranean Countries, and Ruggieri, Gaetano, Observations and Research on Impietratura. *In* W. C. Price (ed.), 2d Conf. Internatl. Organ. Citrus Virol. Proc. 1960, pp. 177–181 and 182–186. 1961.

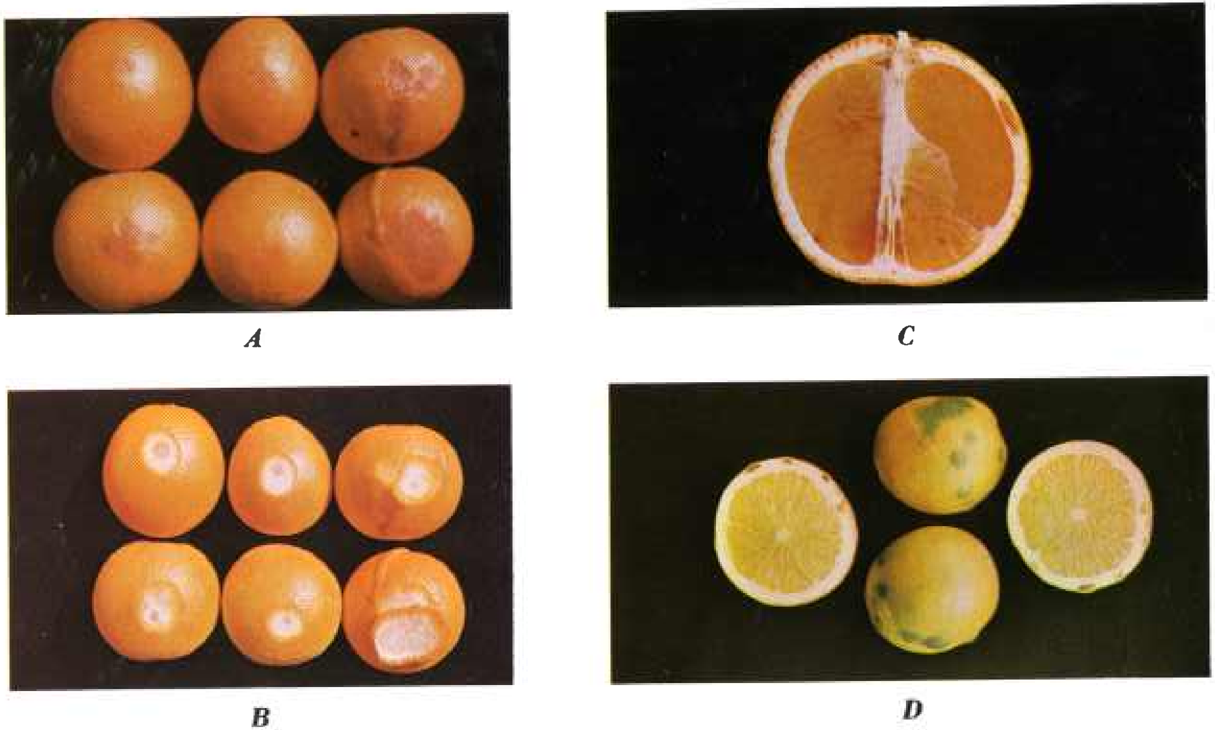

FIGURE 1.—Symptoms of impietratura on sweet orange: *A*, Hard, depressed areas with enlarged oil glands in rind; *B*, same fruits with rind cut to show gum pockets in albedo; *C*, fruit cut to show gum in rind at stylar end, columella, and elsewhere; *D*, green spots on rind resulting from failure of impietratura-affected areas to color normally in ripening. (Courtesy of Henri Chapot and Mortimer Cohen.)

brown, and they may advance outward toward the glandular and interglandular tissue of the flavedo. As seen from the exterior, these tissues appear slightly depressed and contain brown glands that resemble the spots of petechia relata or slight patua. Sometimes the interglandular tissue may not be depressed but rather protruding, and the affected area is well distinguished by the brown color of the single glands as seen from the exterior. By cutting tangentially through the protruding areas, cavities with fluid or coagulated gum up to 1 cc. in volume may be seen in the albedo (fig. 1).

INDEX PROCEDURE [2]

It is advisable to use 2- or 3-year-old grapefruit plants grafted on sour orange (*C. aurantium* L.) as indicator plants. From September through October inoculate on the branches of the young grapefruit trees with bark pieces taken from the candidate tree. Sweet orange also may be used as an indicator plant. The test grapefruit or sweet orange plant must be old enough to produce fruit. A year after inoculation, fruits developing in the spring on the inoculated branches will present characteristic symptoms of impietratura if the candidate tree carried the virus.

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[2] Translated by Victoria Rossetti.

LIKUBIN

By T. Matsumoto, H. J. Su, and T. T. Lo [1]

Likubin has long been known as a nonparasitic disease in Taiwan. However, experiments by the authors since 1956 have shown that this disease is due to a virus closely related to the tristeza virus. Likubin is now generally called huanglunpin, or yellowing of scions, because a disease of similar characteristics was observed on the China mainland and reported by C. P. Cheng (*1*) in 1943 under this name. However, direct comparison is not possible under existing circumstances.

Symptoms

Although the disease syndrome differs somewhat as host varieties differ, the common symptoms are decline in vigor, yellowing or mottling of leaves, premature defoliation, dieback of small twigs, formation of multiple abnormal flowers, decay of feeder rootlets and lateral roots, and wilting of leaves followed by death of the entire plant.

The characteristic symptoms of likubin on several varieties of citrus are as follows: On Ponkan (*Citrus reticulata* Blanco), the leaves of diseased trees exhibit overall chlorosis or yellowing of veins and of mesophyll tissues surrounding the veins (figs. 1 and 2), and less frequently yellowing of the mesophyll tissues with the veins remaining green. This variation in symptom expression may be associated with some environmental conditions or with mineral deficiencies resulting from the virus infection. The vein corking produced on diseased leaves later is apparently similar to the symptom noticed on the Mexican lime (*C. aurantifolia* (Christm.) Swing.) seedlings inoculated with this virus. Young leaves that develop after premature defoliation are usually small and pale green.

Diseased trees tend to blossom early and heavily, but flowers are very small and abnormal, are prematurely shed, and fail to produce fruits. Infected trees occasionally exhibit dieback of branches, yellowing or mottling of leaves, and defoliation on one side, whereas the other side is apparently normal in appearance (fig. 3).

On Tankan (*C. reticulata* Blanco), general decline symptoms are similar to those of the diseased Ponkan, although defoliation usually seems to be quicker for Tankan. On sweet orange (*C. sinensis* (L.) Osbeck), leaf symptoms differ according to variety,

[1] This section was reviewed by J. M. Wallace and L. G. Weathers.

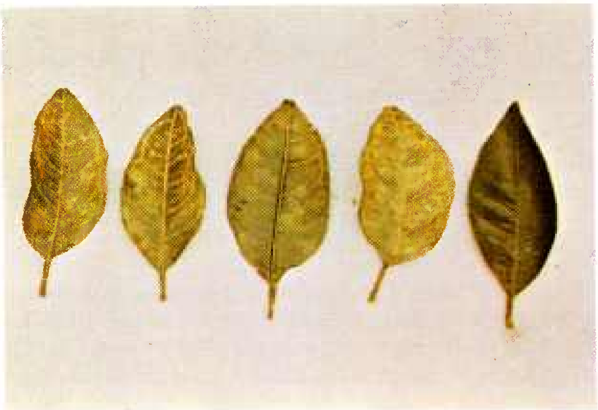

FIGURE 1.—Likubin symptoms on four Ponkan leaves (left) and normal leaf (right).

but for varieties such as Valencia, Sekkan, Washington navel, and Luicheng, the more prominent symptoms are general yellowing or mottling of young leaves, particularly yellowing of veins and adjacent tissue, while the major part of the mesophyll remains green for a considerable time. Stem pitting is observed occasionally in Washington navel.

Symptoms of likubin on the leaves of stocks grafted with infected scions are as follows: On Sunki (*C. reticulata* var. *austera* Swing.), the leaf symptoms are not constant, differing sometimes as the source of scion differs, but the following symptoms are noticed most frequently: The leaves usually exhibit indistinct chlorotic areas between the main lateral veins in from 3 to 4 months, occasionally as early as 2 months after grafting. The chlorotic areas are variable in size and shape, being elongated, circular, or stippled and thus resulting in irregular spotting or mottling with an indistinct margin. This chlorotic discoloration intensifies in time, though rather slowly.

On seedlings of sour orange (*C. aurantium* L.) and of Mexican lime infected by grafting with Ponkan scions, the symptoms are as follows: Sour orange leaves become chlorotic and stunted, usually a few months after grafting, and young leaves of Mexican lime develop dashlike chlorosis of lateral veins of young leaves that can be seen by transmitted light. This symptom progresses into vein clearing followed by vein corking.

The reaction of Eureka lemon (*C. limon* (L.) Burm. f.) to likubin is as follows: A mild virus strain, UO 14, from Valencia orange and a severe virus strain, YMS 15, from Ponkan mandarin were tested with Eureka lemon seedlings (Fraser's test). In both cases, yellowing and stunting of leaves and some cessation of plant growth were noticed on the Eureka lemon seedlings. The

FIGURE 2.—Twigs from likubin-infected (left) and from healthy (right) Ponkan tree.

symptoms on the leaves of these trees inoculated with two different strains were almost the same, presumably similar to the severe type of symptoms described by Fraser (*2*).

Response of stock-scion combinations to the disease is as follows: Trifoliate orange (*Poncirus trifoliata* (L.) Raf.), Cleopatra mandarin (*C. reticulata* Blanco), Troyer citrange (*P. trifoliata* × *C. sinensis*), Yuzu (*C. ichangensis* × *C. reticulata* var. *austera*), *Citrus tachibana* (Makino) Tan., and tangerine (*C. reticulata* Blanco) were used as rootstocks and were mostly topworked with Ponkan scions. The Cleopatra rootstock was rather intolerant to strains of the virus used by the authors, although this rootstock is considered tolerant of tristeza in North America and South America.

Trifoliate orange rootstock was more tolerant than any others tested, which accords with reports from other countries. However, it is noted that this rootstock is by no means absolutely tolerant of all the virus strains existing in Taiwan. For instance, trifoliate orange grafted with Ponkan scion has also been affected, though not seriously, by a newly isolated severe strain YLA, which was

FIGURE 3.—Likubin-infected Ponkan tree partially revealing symptoms.

derived from a diseased Ponkan and proved positive when tested with Mexican lime. It was also found through field surveys and experiments that Rangpur lime (*C. reticulata* var. *austera* hybrid) is highly tolerant of the virus.

TRANSMISSION BY APHIDS

The virus causing likubin was transmitted by *Toxoptera citricida* (Kirkaldy) as follows: Nonviruliferous aphids were fed on diseased citrus trees for 24 hours and then transferred to Mexican lime seedlings (25-70 aphids per plant.) After feeding 24 hours, the aphids were removed and the plants were sprayed with malathion solution (0.02 percent). The young leaves of Mexican lime seedlings infested with viruliferous aphids developed vein clearing in about 40 to 60 days. From these results, likubin disease can be readily transmitted in nature by *T. citricida* from diseased plants to healthy ones.

IDENTIFICATION

In experiments by the authors all the virus isolates derived from the so-called likubin-affected citrus plants proved positive when tested with Mexican lime. Therefore, the virus strains used seem closely related to the tristeza virus prevalent in North America and South America. However, since these virus strains can infect Ponkan on Cleopatra mandarin rootstock, whereas in the aforementioned countries this rootstock is tolerant of the tristeza virus, there is a possibility that the virus contains, besides tristeza, another component that affects Cleopatra. Another difference between these strains and tristeza is the reaction on Sunki.

According to a report submitted to the Joint Commission on Rural Reconstruction by J. M. Wallace (*3*), who visited Taiwan in 1963, there seems to be no doubt regarding the presence of tristeza virus in citrus plantings there. However, he suggested that the Sunki rootstock trees might be declining from the effects of an unknown virus and not from tristeza virus, because in Brazil, trees on Sunki rootstock were reported to be in good condition after many years' exposure to tristeza.

In the experiments by the authors all the isolates derived from the so-called likubin-affected citrus trees growing in Taiwan proved positive when tested with Mexican lime, exhibiting vein clearing and vein corking, and the reactions of the isolates on Eureka lemon were comparable with those of tristeza. On the other hand, the isolates differ from the latter in their reactions against Cleopatra and Sunki. From these data, it is tentatively concluded that the likubin virus is identical with the tristeza virus in its main component, but in addition it contains some other component(s).[2]

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[2] The other component(s) may be greening disease virus or be related to it. C. I. Gonzales, horticulturist, Bureau of Plant Industry, Philippines, reported that pictures received from T. Matsumoto are identical with Philippine leaf mottle disease, which in turn seems to be related to greening disease of South Africa. (J. F. L. Childs)

TATTER LEAF

By J. M. WALLACE and E. C. CALAVAN [1]

Tatter leaf was described in 1962 in California and so named because leaves of infected plants, *Citrus excelsa* Wester (a thick-skinned lime), are distorted and have a torn or ragged appearance. Strong, yellowish leaf mottling and wood pitting or grooving on Troyer citrange (*C. sinensis* (L.) Osbeck × *Poncirus trifoliata* (L.) Raf.) and on certain other trifoliate orange hybrids were attributed later to the tatter leaf virus. However, there is some question as to whether the symptoms described on *C. excelsa* are caused by the same virus as that which produced the disease effects on Troyer citrange. Two different viruses may be involved.

In California the tatter leaf virus was found occurring naturally in Meyer lemon (*C. limon* (L.) Burm. f.) only and was obtained from 14 of 15 Meyer lemon trees tested. Through the citrus variety improvement program and other studies at the Citrus Research Center, Riverside, approximately 400 citrus trees of both foreign and domestic origin were indexed on *C. excelsa* seedlings. None except Meyer lemon were carriers of tatter leaf virus. Most Meyer lemon trees, although symptomless, are carriers of both tristeza and seedling yellows viruses. It is of interest that three Meyer lemon trees free of tristeza and seedling yellows viruses were infected with tatter leaf virus.

CITRUS REACTIONS

Citrus excelsa

Direct inoculations from Meyer lemon to *C. excelsa* result in a blotchy spotting on newly developing leaves that resembles somewhat the effects of psorosis on leaves of lime and lemon. As new leaves form, some are ragged or malformed (fig. 1, *A*). Abnormal leaves may continue to develop generally over the plant for a considerable time, with production of symptomless shoots following later. On other infected plants, a few leaves will show tatter leaf symptoms, but leaves arising subsequently above the affected parts will be normal. *C. excelsa* plants inoculated with pure cultures of tristeza, seedling yellows, xyloporosis, exocortis, psorosis A, crinkly leaf, infectious variegation, and vein enation have not developed the tatter leaf symptoms.

In spite of the fact that *C. excelsa* reacts to infection with seed-

[1] This section was reviewed by L. G. Weathers and S. M. Garnsey.

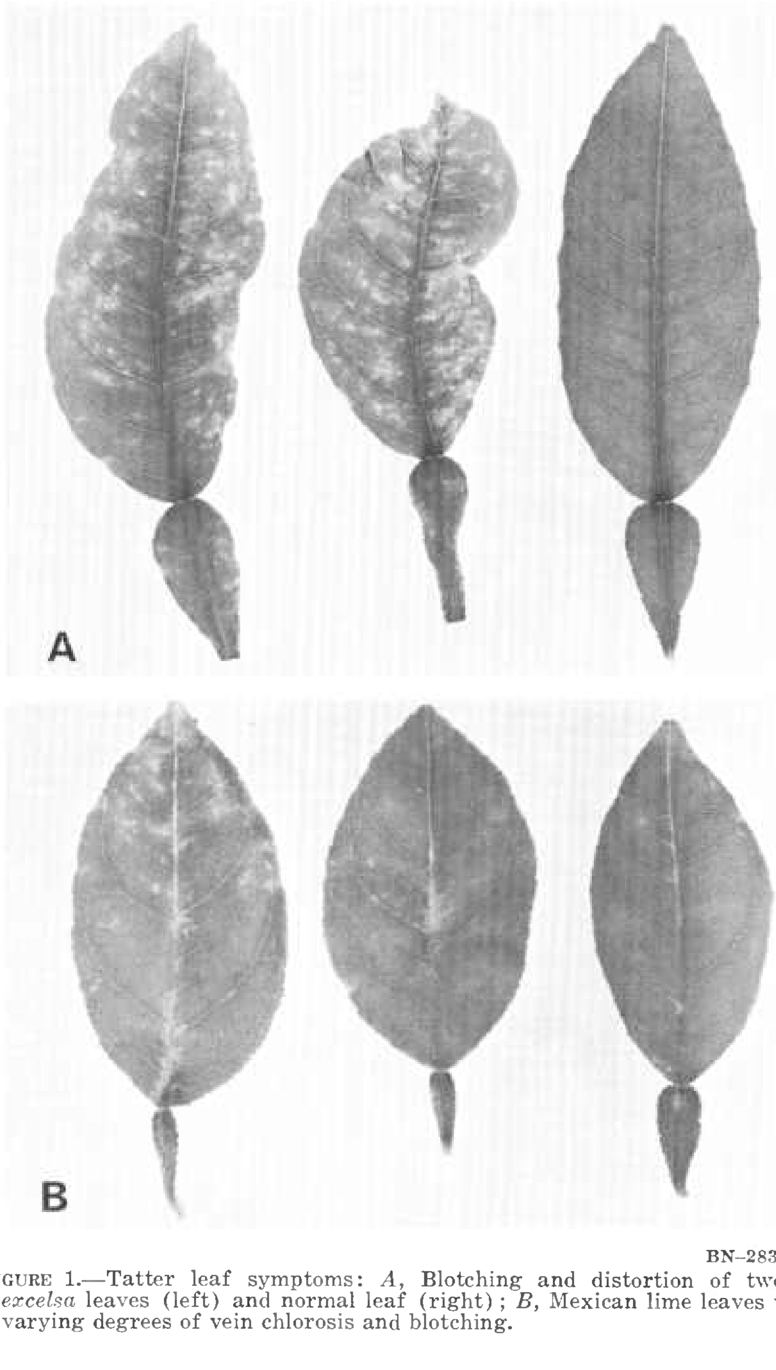

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FIGURE 1.—Tatter leaf symptoms: *A*, Blotching and distortion of two *C. excelsa* leaves (left) and normal leaf (right); *B*, Mexican lime leaves with varying degrees of vein chlorosis and blotching.

ling yellows virus from Meyer lemon trees, tatter leaf symptoms are not suppressed. Occasionally *C. excelsa* and Troyer citrange plants inoculated directly from Meyer lemon fail to develop tatter leaf symptoms. Uneven distribution of the virus in the donor plant or graft failures can explain this.

Lime

Key lime (*C. aurantifolia* (Christm.) Swing.) is not a satisfactory indicator if seedling yellows virus is present. When Meyer lemons carrying both seedling yellows and tatter leaf viruses are indexed on Key lime, the seedling yellows virus tends to mask the symptoms of tatter leaf or the tatter leaf complex.

Sometimes lime seedlings develop no symptoms when inoculated directly from a Meyer lemon that carries only the tatter leaf virus. In other instances leaf symptoms range from slight tristezalike vein clearing to a combination of vein clearing and strong blotching. Parts of the midrib and large veins become cleared or chlorotic and often this chlorosis extends irregularly into the adjacent leaf tissues. These symptoms are shown in figure 1, *B*. Some of the small cleared zones on the lateral veins resemble the vein clearing of tristeza.

However, tatter leaf virus obtained from the Meyer lemon plants free of the seedling yellows virus complex causes no stem pitting on Mexican lime and does not affect trees of sweet orange on sour orange rootstock. Leaf distortion of lime is much less than that of *C. excelsa*. Like *C. excelsa,* lime sometimes screens out the component of the virus mixture that causes symptoms on *C. excelsa.*

Troyer Citrange and Citremon

Troyer and Carrizo citrange and citremon (*P. trifoliata* (L.) Raf. × *C. limon* (L.) Burm. f.) (C.E.S. 1448) react strikingly to infection from Meyer lemons (fig. 2, *A*). Leaf symptoms consist of yellowish mottling, vein banding, and failure of one side of the leaflet to develop, which results in leaf curvature or distortion. At times there is no leaf distortion and the spots or blotches appear as rings. Some sources of the virus cause pronounced stunting. Plants may fail to produce new terminal growth. Superficial lesions that form on the bark of young stems retard the normal development of tissue and cause the stems to grow at an angle. Later the direction of growth changes so as to give the stems a zigzag shape (fig. 2, *B*).

Wood pitting develops on Troyer citrange and citremon, with the individual pits often alined somewhat vertically (fig. 3). With further growth of the trees the pits increase in size and the small vertically arranged pits often coalesce and form continuous grooves visible externally. These effects plus bud-union creasing of trees on susceptible rootstocks later cause decline of the tree.

The possibility that the symptoms produced on *C. excelsa* and on Troyer citrange are caused by two different viruses is based on the observation that after one or two serial passages of the virus from Meyer lemon through *C. excelsa,* the virus remaining pro-

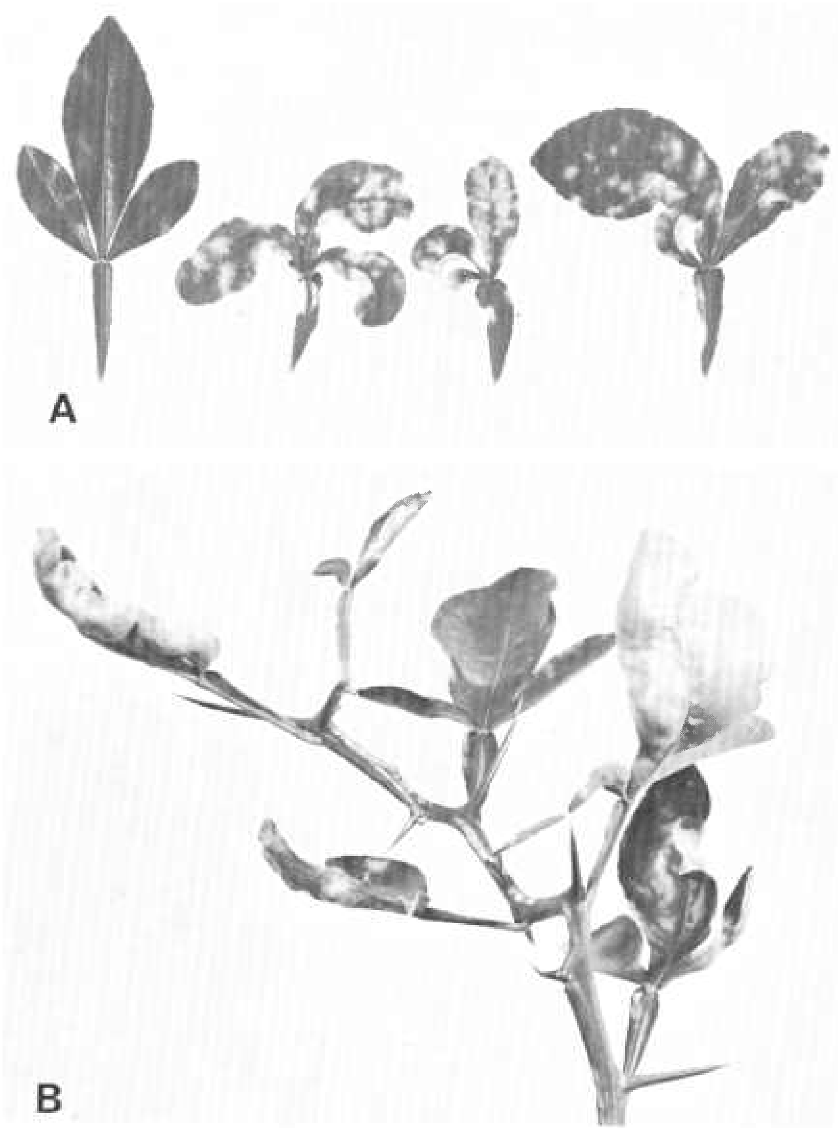

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FIGURE 2.—Tatter leaf symptoms: *A*, Normal (left) and three affected leaves (right) of citremon; *B*, leaf distortion, corky lesions on stems, and irregular stem growth of Troyer citrange.

duces a reaction on Troyer citrange but no longer causes any symptoms on *C. excelsa*. This situation is being investigated further.

Whether or not this virus or virus complex exists anywhere in

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FIGURE 3.—Pitting on stems of citremon infected with tatter leaf virus.

field citrus trees other than Meyer lemon is not established, but the severe effects caused on the widely used Troyer citrange rootstock by the virus or virus mixture in Meyer lemon make it advisable to include proper indicators for it in all citrus virus indexing programs. Until the matter of virus mixture is settled, these indicators should be *C. excelsa* and a susceptible citrange or citremon. Seeds of citranges are doubtless available to most workers. Both Troyer and Carrizo citrange are good indicators.

Host Range and Symptoms

Apparently the tatter leaf virus or virus complex has a wide host range among citrus species and varieties. In limited studies both suspected components were recovered from experimentally inoculated sweet orange (*C. sinensis* (L.) Osbeck), sour orange (*C. aurantium* L.), lemon (*C. limon* (L.) Burm. f.), and Rough lemon (*C. limon* (L.) Burm. f.), although all of them remained symptomless. Trifoliate orange (*P. trifoliata* (L.) Raf.) has only rarely developed symptoms and appears to be a poor host. After showing true tatter leaf symptoms, *C. excelsa* commonly recovers by producing normal foliage. Inoculations from such plants result in symptoms on Troyer citrange, but usually fail to reproduce the tatter leaf effects on *C. excelsa.*

Indexing Procedures

The usual procedures should be followed in indexing for tatter leaf virus. Seedlings of indicator varieties should be grown and inoculated by tissue grafts as in other citrus virus indexing. It is advisable perhaps to include *C. excelsa* plus either Troyer or Carrizo citrange as indicators.[2] As yet, no direct inoculations from Meyer lemon have caused the *C. excelsa* reaction while failing to affect the citranges. It is after passage through *C. excelsa* that transfers to *C. excelsa* usually fail to result in symptoms, whereas the citranges continue to develop strong symptoms.

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[2] Garnsey finds many Meyer lemon trees in Florida that do not carry tatter leaf virus, although some were indexed three times. However, tatter leaf-negative trees carry tristeza virus, as Wallace observed in California. Garnsey finds Rusk citrange (*C. sinensis* (L.) Osbeck × *P. trifoliata* (L.) Raf.) a good indicator plant, better than Troyer and much better than Carrizo. (J. F. L. Childs)

LEAF CURL

By Ary A. Salibe [1]

A leaf-curling disease affecting some Hamlin and Pera sweet orange trees was found in the Limeira area in Brazil. This disease is of virus nature and inoculation tests show that it can affect trees of almost all commercial citrus varieties and species, independent of the rootstock. No vector is known for the leaf curl virus. Affected trees decline and remain completely unproductive or die. All trees with symptoms resembling those here described should be tested and destroyed immediately if positive results are obtained. Testing takes from 2 to 5 months.

Symptoms

Early symptoms of leaf curl usually appear on one to several branches of a normal tree. The leaves of the infected branches first show vein clearing, then turn yellow, and usually drop. Sprouts grow abundantly from these branches. The new leaves are small and curled in a manner suggestive of heavy infestation by aphids (fig. 1). Later the symptoms progress to other branches and then to the whole tree. Finally the branches die back, and normal leaves sprouting from the trunk give the diseased tree a gross appearance somewhat similar to that described for "blight" in Florida. The young sprouts are weak, frequently have gum in the wood vessels, and break off readily under slight pressure. Blooming is abundant, but only a few flowers develop into fruits and they are small. The seeds of these fruits produce normal seedlings (*1*).

The leaf curl virus affects trees of sweet orange (*Citrus sinensis* (L.) Osbeck), sour orange (*C. aurantium* L.), tangerine (*C. reticulata* Blanco), lemon (*C. limon* (L.) Burm. f.), acid and sweet limes (*C. aurantifolia* (Christm.) Swing.) except Rangpur lime (*C. reticulata* var. *austera* hybrid), grapefruit (*C. paradisi* Macf.), shaddock or pummelo (*C. grandis* (L.) Osbeck), and citron (*C. medica* L.). Symptoms of the disease develop similarly in trees of all these species, but are more striking in the sweet orange varieties. Infection with other viruses, such as tristeza, exocortis, and psorosis, does not protect the tree from the establishment of the leaf curl virus. Symptoms are expressed in young trees from 2 to 5 months after inoculation whereas incubation in

[1] This section was reviewed by S. Moreira and Victoria Rossetti.

FIGURE 1.—Bahianinha navel orange tree on Caipera sweet orange rootstock affected by leaf curl virus at Limeira Experiment Station, Brazil, in 1963.

old trees may take 1 to 2 years. Pruning hastens the appearance of disease symptoms (*2*).

INDEXING PROCEDURE

Nursery seedlings of common sweet orange, 1 year old, are used as indicator plants for the leaf curl virus. Budwood is taken from the trees to be tested and is budded in the test seedlings. It is advisable to use five seedlings for each tree to be tested and to insert many buds in each one of them. This recommendation is based on the fact that the leaf curl virus is unevenly distributed in the infected trees (*1, 2*). Twenty days later, if the inoculation buds are still alive, the sweet orange seedlings are severely pruned and sprouts are allowed to grow.

In the presence of the leaf curl virus, the leaves of the young sprouts are small, curled, and show vein clearing. Sometimes the first leaves are normal and the following ones are affected by the virus. Symptoms usually appear within 2 to 5 months after inoculation. Dieback of the young sprouts and death of the seedling both occur frequently. Symptoms are more conspicuous from inoculations made in late autumn or spring.

If sweet orange seedlings are not available, testing can still be carried out by using seedlings of other citrus species. The seedlings should be similarly inoculated with buds from the tree to be tested, and subsequently budded above the inoculation with a bud from a healthy sweet orange that will serve as an indicator. Each test seedling is then cut back and the sweet orange bud is allowed to sprout. Sprouts should be examined for leaf curl virus symptoms. Positive results should be promptly followed by eliminating the diseased trees. Under favorable environmental conditions, normal and vigorous sprouting of the indicator plants up to 12 months after inoculation is strong evidence of the absence of the leaf curl virus in the test tree.

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PODAGRA

By L. C. KNORR [1]

Podagra is a disorder of Rough lemon (*Citrus limon* (L.) Burm. f.) rootstocks, which enlarge to resemble trifoliate orange (*Poncirus trifoliata* (L.) Raf.) rootstocks and exhibit exocortislike scaling of the bark (fig. 1).

Podagra is found in Florida wherever certain clones of Nagami (*Fortunella margarita* (Lour.) Swing.) and Meiwa kumquats (*F. crassifolia* Swing.) have been budded on Rough lemon. The disease does not appear when the same clones are budded on Cleopatra mandarin (*C. reticulata* Blanco), sweet orange (*C. sinensis* (L.) Osbeck), and sour orange (*C. aurantium* L.) (*1, 2*).

Podagra's economic importance lies less in its damage to kumquat plantings than in its possible consequences to Rough lemon, the rootstock on which approximately 75 percent of Florida's citrus is grown.

Podagra-affected trees in some kumquat groves on Rough lemon rootstock amount to 50 percent. Despite such losses, growers still prefer to grow kumquat trees on Rough lemon stocks because of the lower solids produced in the fruits.

SYMPTOMS

The name "podagra," from the Greek *pous* (foot) plus *agra* (seizure), refers to the swollen rootstock part of the trunk. Overgrowth is accompanied by scaling of the Rough lemon bark (fig. 1). In the spring profuse gumming often accompanies the shelling of bark. At first these symptoms suggest that the trees are budded on trifoliate orange affected by exocortis. This erroneous impression is sustained by the exocortislike stunting and decline shown in affected tops. Whether affected trees carry exocortis virus is not known at the present time.

Symptoms of podagra may appear as early as 1 year after budding or as late as 10 years after setting of trees in the grove. Speed of deterioration varies; some trees succumb in the nursery, others decline gradually in the field.

TRANSMISSION TESTS

The possibility that podagra is a virus disease is supported by

[1] This section was reviewed by J. F. L. Childs.

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FIGURE 1.—Overgrowth and scaling of Rough lemon rootstock part of kumquat tree affected by podagra.

preliminary results of transmission tests begun in 1957. Results showed that budwood taken from affected trees reproduced podagra when budded into Rough lemon seedlings. On the other hand, trees made from budwood of normal trees have not developed podagra 8 years after budding.

Transmission tests in the field include test plants with interstocks of sweet orange, sour orange, Rangpur lime (*C. reticulata* var. *austera* hybrid), and citron (*C. medica* L.) to determine whether the hypothetical virus in kumquat tops will pass through symptomless tissue and affect Rough lemon.

Indexing Procedure

No more critical procedure for indexing is known than the inoculation of Rough lemon seedlings with buds from suspect kumquat trees. The first symptom, formation of small pegs from the cambial face of the wood, may be seen by peeling a patch of bark. This occurs about 2 years after inoculation. Gumming and scaling develop at about 4 years. Field trees commence to decline after 4 years.

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INDEXING FOR TATTER LEAF WITH COWPEA

By S. M. GARNSEY and L. G. WEATHERS

A virus present in Meyer lemon (*Citrus limon* (L.) Burm. f.) has been transmitted by sap inoculation to several herbaceous plants including cowpea (*Vigna sinensis* (Torner) Savi) (*2, 3*). It was suggested that this sap-transmissible virus was tatter leaf virus (*2*), and recent transmission experiments by Weathers (unpublished data, 1966) have confirmed this.

INDICATORS

Tests conducted in Florida show that the Early Ramshorn variety of cowpea is a reliable and rapid indicator for tatter leaf virus (TLV) when inoculated properly. Forty-three Meyer lemon trees have been indexed for TLV by (1) bud-inoculating seedlings of Rusk citrange (*C. sinensis* (L.) Osbeck × *Poncirus trifoliata* (L.) Raf.) and (2) inoculating cowpea plants with macerated tissue of young citrus leaves made by grinding the leaves in cold neutral phosphate buffer. The results correlated exactly. Twenty-eight trees tested positively on both Rusk citrange and cowpea, whereas the remaining 15 tested negatively.

TLV was also transmitted readily to cowpea from infected seedlings of sweet orange (*C. sinensis* (L.) Osbeck), Duncan grapefruit (*C. paradisi* Macf.), Cleopatra mandarin (*C. reticulata* Blanco), Orlando tangelo (*C. paradisi* × *C. reticulata*), Eureka lemon (*C. limon* (L.) Burm. f.), *C. excelsa* Wester, and sour orange (*C. aurantium* L.). It was readily transmitted from leaflets of Rusk or Troyer citrange that showed symptoms of TLV infection, but not from leaflets that were symptomless. TLV was transmitted inconsistently from Mexican lime (*C. aurantifolia* (Christm.) Swing.).

SYMPTOMS

The diagnostic symptom in cowpea consists of spreading necrotic lesions on the inoculated primary leaves (fig. 1). The lesions are chocolate brown and easily visible 4 to 6 days after inoculation, provided the inoculated plants are held at moderate temperatures (near 70° F.). High temperatures markedly reduce symptom severity (fig. 1). Under certain conditions mosaic patterns appear in the trifoliate leaves.

INDEXING PROCEDURE

Macerate young succulent leaf tissue in cold neutral potassium phosphate buffer (0.05 molar), using a prechilled porcelain mortar and pestle. A ratio of 0.1 gram of leaf tissue to 1 ml. of buffer is satisfactory. Using a finger or cotton swab, gently apply the macerate to the primary leaves of cowpea that have been dusted with 500-mesh carborundum (passes a screen of 500 holes per square inch). After inoculation, rinse the leaves with cool tap-water and incubate the inoculated plants under moderate light (500 to 1,000 foot-candles) at 21° to 24° C.

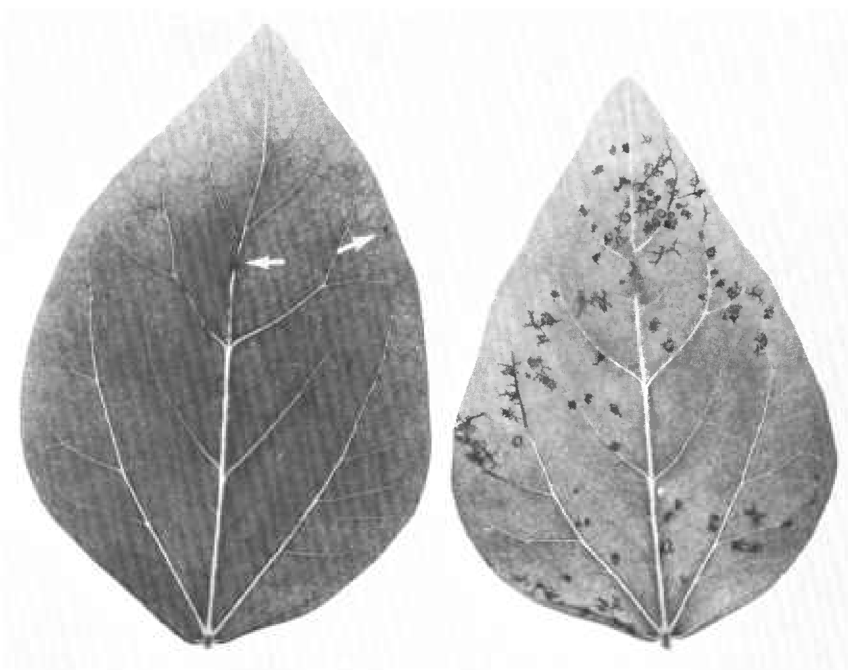

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FIGURE 1.—Inoculated leaves of cowpea: *Left*, incubated at summer greenhouse temperatures of 70°–90° F.; *right*, held at 70° ±2°; light conditions were similar. Note small lesions (arrows) on leaf at left.

Although there is no established rule on the number of plants required, experience indicates that from 5 to 10 plants should be used for each test. Cowpea plants are easily grown by planting from six to eight seeds in 4-inch clay pots filled with steam-sterilized potting soil. The seedlings are thinned to obtain four or five uniform plants per pot. Inoculate when the primary leaves have expanded, but before the trifoliate leaves appear. The plants are usually ready to inoculate 8 to 12 days after planting depending on growing conditions.

This procedure consistently produced reliable results, even when indexing leaf tissue collected in the field in midsummer. However, it is well to note that a sap-inoculation technique requires higher concentration of virus in the inoculum than is necessary for graft transmission and to that degree is a less sensitive procedure. Citrange test plants also should be graft inoculated from the same inoculum source until it is established that the cowpea test gives reliable results under local conditions. The viruses of tristeza, exocortis, xyloporosis, psorosis, stubborn, vein enation, and yellow vein have not produced symptoms on cowpea. Infectious variegation virus will infect several varieties of cowpea (*1*), but the symptoms are easily distinguishable from those produced by TLV.

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COLOR TEST FOR EXOCORTIS

By J. F. L. Childs

Several improvements in indexing for exocortis have been made since the color test (*2*) was reported. The Rangpur lime (*Citrus reticulata* var. *austera* hybrid) test (*3*) has given good results under certain conditions, but it has proved slow and sometimes erratic in Florida. The Etrog citron (*C. medica* L. var. *ethrog* Engl.) test (*1*), using certain selections of citron, has given excellent and rapid results. However, occasions arise when the color test can be used to advantage.

The use of radial-longitudinal sections of bark instead of cross sections, as first reported, makes diagnosis by the color test more certain and easier. Best results with the color test are obtained with trifoliate orange (*Poncirus trifoliata* (L.) Raf.), but the following citranges (*P. trifoliata* (L.) Raf. × *C. sinensis* (L.) Osbeck and reciprocal) are satisfactory: Carrizo, Morton, Savage, and Norton. The Rusk, Rustic, Thomasville, and Uvaldi citranges react weakly.

The color test indicates an early stage of bark scaling that occurs internally from 6 months to several years before scaling is apparent to the eye. Consequently, trees on exocortis-susceptible rootstocks can be examined at an early age by this method, or seedlings of exocortis-susceptible varieties can be inoculated with buds or grafts for the test.

Procedure

A small piece of bark, approximately 5 by 30 mm., is cut from the test tree anywhere between the bud union and the ground line. In experiments at Orlando, Fla., better results were obtained on the average with bark cut near the union. Specimens can be examined fresh or stored in standard formaldehyde, alcohol, or acetic acid fixing solution for examination later.

The outer surface of the bark should be washed with a brush to remove adhering dirt, sand, or algae. Mount the specimen on a freezing microtome so that sections can be cut in the radial-longitudinal plane and so that the blade enters the specimen on a slight diagonal from the cambial side. This avoids dragging sand across the section. A clinical freezing microtome is satisfactory for this purpose. A sliding microtome with freezing attachment is better, but more expensive.

Microtome blades must be very sharp to avoid tearing the sections, which are cut at 18 to 20μ. Since blades are soon damaged by sand adhering to the bark, examine the blade after cutting each specimen of bark. If it is damaged, change to a sharp part of the blade to cut the next specimen. After 20 or more good sections have been cut, mount 5 to 10 of the best ones on a glass microscope slide in rows of 5 sections each. It is suggested that 15 sections be examined from each bark specimen.

Arrangement of the sections is facilitated if they are moved into position on the glass slide in a film of water. Drain off excess water and blot the remaining moisture by laying a piece of filter paper over the sections and running a finger lightly over the paper. A few drops of phloroglucinol (saturated solution in 18 percent hydrochloric acid (HCl)) are allowed to run down the slide between the sections so that all are wet by it. This solution keeps for several months, but solutions 6 months or older may fail to give the color test. A cover slip, 22 by 60 mm. (preferably No. 1, 0.15-mm. thickness), is placed over the sections, bubbles are pressed out, and the edges sealed with vas-par (equal parts melted paraffin wax and petroleum jelly) to keep HCl fumes from the microscope. After 5 minutes the section may be examined. After examination, the slides should be kept in an airtight container until disposed of so as to keep HCl fumes away from instruments.

Diagnosis

Examine only the medullary ray cells of the phloem region. The advantage of radial-longitudinal sections over cross sections is that, in the former, medullary ray cells are more easily distinguished from the sieve tubes. In the radial-longitudinal plane, the rectangular ray cells are arranged with the long axis of the cell at right angles to the cambium. All other cells in the phloem region are arranged with their long axis parallel to the cambium. Also, medullary rays are 6 to 30 cells wide in the radial-longitudinal plane compared to the transverse plane where rays are only a few cells wide.

Exocortis virus causes the formation of an aldehyde in some of the ray cells. This aldehyde develops a rose red (pomegranate purple by Ridgeway's color standards (*4*)) when treated with phloroglucinol-HCl reagent (figs. 1 and 2). Any test for an aldehyde will work, but the phloroglucinol-HCl test proved most satisfactory. The color should be distributed uniformly throughout the cytoplasm of the cell. Not all ray cells develop the color. A positive reaction can not be expected sooner than 6 months after inoculation, but may be delayed much longer if growth of the test plant is poor. Ray cells occasionally develop a light pink (Cameo pink, Ridgeway (*4*)), which has no known relation to the exocortis virus.

As an arbitrary standard, the author has held that if three ray cells per slide develop the pomegranate purple, the exocortis test is positive. In practice this should be confirmed by examining at least 15 sections. Ray cells with red-stained cytoplasm are frequently so numerous that there is no need to examine more than five sections. To gain experience with the method and as a basis for comparison, examination of bark known to be exocortis infected is recommended.

FIGURE 1.—Medullary ray cells oriented in horizontal rows; sieve tubes and phloem fibers in vertical rows. Note rose-red cells in group of ray cells.

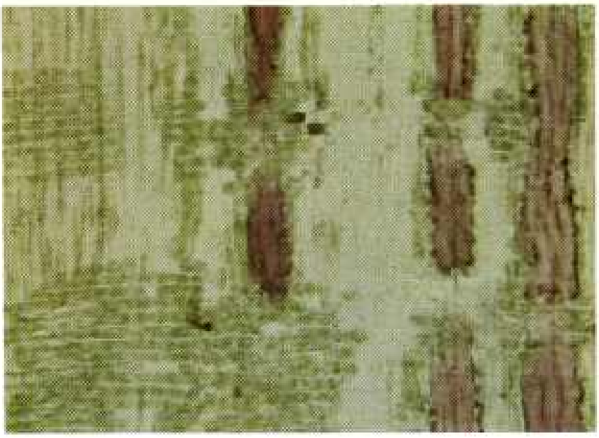

FIGURE 2.—Two stained cells in one medullary ray and one in other ray is typical of young *P. trifoliata* bark infected with exocortis.

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GREENING DISEASE

By Ralph E. Schwarz

Greening disease has been known for more than 35 years in South Africa, but it was proved to be a virus disease only recently (*1, 2*). Transmission of the causal virus by the citrus psylla *Spanioza erythreae* (Del Guercio) was established by McClean and Oberholzer (*1*). The world distribution of this disease has not been fully determined, but recent information indicates that greening or a closely related virus disease is common in several Asian countries and present in the Mediterranean area. Greening disease presents a more serious problem than any other known virus disease of citrus, because it affects the major citrus species irrespective of rootstock variety, reducing yields and making many fruits worthless.

Symptoms

A complete description of the symptoms of greening disease on all commercially important varieties of citrus is not possible here. The symptoms on sweet orange are described in detail, and those on other varieties are noted when they differ significantly.

Greening alters the growth habit or shape of sweet orange trees and causes symptoms on the leaves and fruits, but the best diagnostic symptoms appear on the foliage of seedling trees. Leaves that develop after inoculation show chlorotic patterns similar to those of zinc deficiency. The leaves may be completely yellow or yellow with green veins and small green spots. Mature leaves often show yellowing of the midrib, which becomes abnormally prominent. This condition may extend along the lateral veins and ultimately involve the whole leaf. Mature leaves may also show mottling or blotching (fig. 1). Excessive blooming and out-of-season blooming occur frequently on orchard trees and may be followed by abnormal dropping of fruit and dieback of twigs. Mature fruits may color only on the side exposed to the sun while the unexposed side remains a dull olive green. Only a part or even a single branch of an orchard tree may be affected. Furthermore, the virus seems to be distributed unevenly in the tissues of infected trees.

Leaf and fruit symptoms are masked under certain climatic conditions. For example, when inoculated seedlings are kept at 21° to 23° C., clear leaf symptoms develop, but if infected seedlings are maintained at 26° to 28°, no symptoms appear on the new growth (fig. 2). Although further information on the effects of climate on symptoms is not available, other environmental effects are suspected.

Equally severe symptoms are exhibited by many mandarin, tangelo, and grapefruit varieties (fig. 3). On lemon varieties symptoms are less severe. Very mild symptoms appear on Palestine sweet lime, West Indian lime, Rough lemon, and trifoliate orange and its hybrids.[1]

INDEXING PROCEDURE

Indicator Plants.—Either sweet orange or tangelo seedlings may be used as indicator plants. In the absence of seedling yellows virus, grapefruit seedlings may be used. If there is a choice, it would be Valencia, midseason orange varieties, and navel orange in that order. For tangelos, the order would be Orlando, Minneola, and Seminole. Others may serve as well, but they have not been used.

Preparation of Test Plants.—Seedlings for indexing greening virus should be grown and inoculated as described for psorosis A virus. Because acute symptoms of zinc deficiency are similar to the symptoms produced by greening virus, test plants should be adequately supplied with the minor elements, especially zinc. A University of California type of potting mixture may be used.

[1] For detailed descriptions of greening symptoms on many varieties of citrus, refer to McClean (*1*) and Schwarz (*2*, *3*).

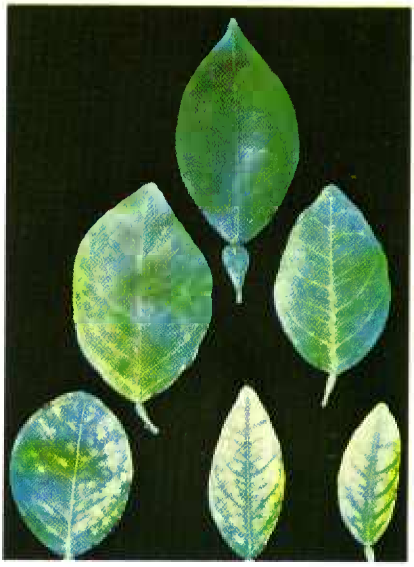

FIGURE 1.—Greening symptoms on sweet orange leaves: Top, normal; middle row, yellow vein; bottom row, mottling and zinc deficiency.

FIGURE 2.—Greening symptoms on sweet orange present on lower leaves kept at low temperature, but absent from top leaves maintained at high temperature.

Inoculation Methods.—Graft sticks from mature wood having leaves with symptoms should be used for inoculation. Sticks are preferable to buds or bark patches, because the virus seems to be unevenly distributed in the host and may otherwise be missed.

Since the percentage of successful transmissions is often low, about 20 test plants are recommended for each test. Citrus varieties used for indexing seedling yellows virus such as lemon and grapefruit are also susceptible to greening disease, and the symptoms produced on such plants by the two viruses are difficult to distinguish. It is therefore not advisable to use them as indicators in the presence of seedling yellows virus.

Diagnosis.—Typical leaf symptoms usually show up on the new growth 3 to 4 months after inoculation. The same range of leaf symptoms observed in orchard trees can usually be produced on test plants in the greenhouse.

The presence of a specific fluorescent marker substance in the tissue of greening-infected sweet orange, tangelo, and mandarin trees can be used for indexing purposes. The albedo of sweet orange fruits from infected trees fluoresces under ultraviolet light of wavelength 365 mμ (*3*). This substance can be extracted from the albedo and bark of citrus and its presence determined by chromatographic methods (*3*).

FIGURE 3.—Greening symptoms on Empress mandarin leaves.

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CLASSIFICATION OF CITRUS SPECIES [1]

By Philip C. Reece

W. T. Swingle recognized 16 species in the genus *Citrus,* in what he considered an approach toward a natural system of classification based on sexually inherited similarities. Borderline and intermediate forms he considered hybrids and placed as hybrids with the species they most closely resembled.

T. Tanaka recognized 150 species in a pragmatic system of classification and assigned Latin binomials to obvious hybrids, apomictically self-perpetuating forms, as well as types that can only be perpetuated by budding or other vegetative means of propagation. Even a single individual tree is given species designation. Thus, he considers the Temple orange, Meyer lemon, Tahiti lime, and other cultivars to be valid species.

Whether one prefers either of these systems of classification or takes R. W. Hodgson's intermediate position, which is mainly the Swingle classification with an additional 23 species of Tanaka, the fact remains that more than one system is in use.

In the following list are given the technical binomials of the Tanaka systematics of the genus *Citrus,* the common name, and the technical binomial equivalent used in the Swingle systematics:

Tanka Citrus *species name*	*Common name*	*Swingle* Citrus *species equivalent*
alata Tan.	Winged citron	*medica* L.
amblycarpa Ochse	Djerock limoo	*reticulata* Blanco
ampullacea Hort. ex Tan.	Hyokan	*paradisi* Macf. (?)
anonyma Hort. ex Tan.	Shirenbo	*paradisi* Macf.
asahikan Hort. ex Tan.	Asahikan	*paradisi* Macf.
assamensis Dutta et Bhatt.	Assam lemon	*limon* (L.) Burm. f.
aurantifolia (Christm.) Swing.	Lime	*aurantifolia* (Christm.) Swing.
aurantium L.	Sour orange	*aurantium* L.
aurata Risso	Adam's apple	*limon* (L.) Burm. f.
aurea Hort. ex Tan.	Kawabata-mikan	*sinensis* (L.) Osbeck
balincolong Tan.	Balincolong	*micrantha* Wester (?)
balotina Poit. et Turp.	Balotin bergamot	*limon* (L.) Burm. f.
benikoji Hort. ex Tan.	Benikoji, Dai Koji	*reticulata* Blanco

[1] Based on material from Swingle, W. T., and Reece, P. C., The Botany of Citrus and Its Wild Relatives of the Orange Subfamily. *In* Reuther, W., Webber, H. J., and Batchelor, L. D. (eds.), The Citrus Industry, 2d ed., v. I, ch. 3, Univ. Calif. Div. Agr. Sci., Berkeley. 1967.

bergamia Risso et Poit.	Bergamot	*aurantium* (L.)
boholensis Tan.	Bohol papeda, Kansi	*micrantha* Wester (?)
canaliculata Hort. ex Y. Tan.	Kiku-daidai	*aurantium* L.
celebica Koord.	Celebes papeda	*celibica* Koord.
clementina Hort. ex Tan.	Clementine	*reticulata* Blanco
combara Raf.	Annam papeda	*macroptera* var. *annamensis* Tan.
crenatifolia Lush.	Kawla, Keonla	*reticulata* Blanco
davaoensis Tan.	Davao lemon	*aurantifolia* (Christm.) Swing.
deliciosa Ten.	Willowleaf mandarin	*reticulata* Blanco
depressa Hayata	Shekwasha	*reticulata* Blanco
el-kantara Y. Tan.	El-kantara	*limon* (L.) Burm. f.
erythrosa Hort. ex Tan.	Fukushu, Kobeni	*reticulata* Blanco
excelsa Wester	"Le Nestour," Limon Real	*aurantifolia* (Christm.) Swing.
flavicarpa Hort. ex Tan.	Maeda-kan	*paradisi* Macf.
funadoko Hort. ex Y. Tan.	Funadoko-mikan "like Temple"	*sinensis* (L.) Osbeck
genshokan Hort. ex Tan.	Genshokan	*reticulata* Blanco
glaberrima Hort. ex Tan.	Kinukawa-mikan	*paradisi* Macf.
grandis (L.) Osbeck	Pummelo, Shaddock, Jabon, Zabon	*grandis* (L.) Osbeck
hainanensis Tan.	Hainan wild orange	*reticulata* Blanco
hanaju Sieb. ex Shirai	Hanayu, Tokoyu	*ichangensis* Swing.
hassaku Hort. ex Tan.	Hassaku orange	*paradisi* Macf.
himekitsu Hort. ex Tan.	Himekitsu	*paradisi* Macf.
hiroshimana Hort. ex Y. Tan.	Natsuzabon	*paradisi* Macf.
hyalopulpa Tan.	Sekiroku-kan	*aurantifolia* (Christm.) Swing.
hystrix DC.	Mauritius papeda, Porcupine orange	*hystrix* DC.
ichangensis Swing.	Ichang papeda	*ichangensis* Swing.
indica Tan.	Indian wild orange	*indica* Tan.
inflata Hort. ex Tan.	Kizu, Mochiyu	*paradisi* Macf.
intermedia Hort. ex Tan.	Yama-mikan	*aurantium* hybrid
iwaikan Hort. ex Tan.	Iwaijabo	*paradisi* Macf.
iyo Hort. ex Tan.	Iyo-mikan	*sinensis* (L.) Osbeck
jambhiri Lush.	Rough lemon	*limon* (L.) Burm. f.
javanica Blume	Djerock Hunje	*aurantifolia* (Christm.) Swing.
junos Sieb. ex Tan.	Yuzu	*ichangensis* × *C. reticulata* var. *austera* (?) hybrid
karna Raf.	Karna, Khatta, Id lemon	*limon* (L.) Burm. f.
keraji Hort. ex Tan.	Keraji	*reticulata* Blanco

kerrii Tan.	Kerr's Thailand papeda	*macroptera* var. *kerrii* Swing.
kinokuni Hort. ex Tan.	Kinokuni, Kishu-mikan	*reticulata* Blanco
kotokan Hayata	Kotokan-tiger head	*paradisi* Macf.
latifolia Tan.	Persian lime, Tahiti lime	*aurantifolia* hybrid
latipes (Swing.) Tan.	Khasi papeda	*latipes* (Swing.) Tan.
leiocarpa Hort. ex Tan.	Koji	*reticulata* Blanco
limetta Risso	Mediterranean sweet lemon	*limon* (L.) Burm. f.
limettioides Tan.	Sweet lime of India	*aurantifolia* (Christm.) Swing.
limon (L.) Burm. f.	Lemon	*limon* (L.) Burm. f.
limonia Osbeck	Rangpur lime	*reticulata* var. *austera* hybrid
limonimedia Lush.	Bajoura, Etrog citron	*medica* L. var. *ethrog* Engl.
longelimon Tan.	"too long to be a lemon"	*limon* (L.) Burm. f.
longispina Wester	Tamisan, Talamisan	*aurantifolia* (Christm.) Swing.
lumia Risso	Lumie, Lumia of Italy	*limon* (L.) Burm. f.
luteo-turgida Tan.	(unknown)	*sinensis* (L.) Osbeck
lycopersicaeformis Hort. ex Tan.	Cleopatra	*reticulata* Blanco
macrophylla Wester	Colo, Alemow	*celebica* Koord. hybrid
macroptera Mont.	Melanesian papeda, Cabuyao	*macroptera* Mont.
maderaspatana Hort. ex Tan.	Guntur sour	*aurantium* L.
madurensis Lour.	Shikikitsu, Tokinkan, Calamondin, Shikinari-mikan	*reticulata* var. *austera* (?) $\times$ *Fortunella* sp.
mairay Wester	Mairay	*aurantium* hybrid
medica L.	Citron	*medica* L.
medioglobosa Hort. ex Tan.	Naruto orange, Naruto-mikan	*paradisi* Macf.
megaloxicarpa Lush.	Amilbed, Keem citron	*limon* (L.) Burm. f. (?)
mellarosa Risso	Mellarosa of France	*limon* (L.) Burm. f.
meyerii Y. Tan.	Meyer lemon	*limon* $\times$ *sinensis*
micrantha Wester	Biasong, small flowered papeda	*micrantha* Wester
mitsuharu Hort. ex Y. Tan.	Mitsuharu-mikan	*paradisi* Macf.
montana Tan.	Bilolo	*aurantifolia* (Christm.) Swing.
myrtifolia Raf.	Myrtle leaf orange, Chinnoto	*aurantium* var. *myrtifolia* Ker.-Gawl.
nana Tan.	Small globose citron, Limau susu	*medica* L.
natsudaidai Hayata	Daidai-mikan, Japanese summer grapefruit	*paradisi* hybrid (?)
nippokoreana Tan.	Korai-tachibana	*sinensis* (L.) Osbeck

nobilis Lour.	King, Kunenbo	*sinensis* × *reticulata* hybrid
oblonga Hort. ex Y. Tan.	Washington navel, Daenkan elliptic orange	*sinensis* (L.) Osbeck
obovoidea Hort. ex Takahashi	Kinkoji	*paradisi* Macf.
obversa Hassk.	Djerock Balik of Java	*aurantifolia* (Christm.) Swing.
odorata Wester ex Tan.	Tihi-tihi, flat citron	*medica* L.
oleocarpa Hort. ex Tan.	Yu-pi-chieh	*reticulata* Blanco
omikanto Hort. ex Y. Tan.	Omi-Kanto	*paradisi* Macf.
otachibana Hort. ex Y. Tan.	Daikitsu, Otachibana	*paradisi* Macf.
oto Hort. ex Y. Tan.	Oto-mikan	*reticulata* Blanco
ovata Hassk.	Djerock tanjoeng boerrah	*aurantifolia* (Christm.) Swing.
panuban Tan.	Panuban	*grandis* (L.) Osbeck
papaya Hassk.	Djerock papaya	*aurantifolia* (Christm.) Swing.
papillaris Blanco	Tizon	*aurantium* hybrid
paradisi Macf.	Grapefruit	*paradisi* Macf.
paratangerina Hort. ex Tan.	Ladoo	*reticulata* Blanco
pennivesiculata Tan.	Gajanimma, Moi	*limon* hybrid (?)
peretta Risso	Peretta of France	*limon* (L.) Burm. f.
platymamma Hort. ex Tan.	Saagkam	*reticulata* Blanco
ponki Hort. ex Tan.	Ponki	*reticulata* Blanco
pseudoaurantium Hort. ex Y. Tan.	Henka-mikan	*ichangensis* hybrid
pseudograndis Hort. ex Tan.	Uzon-kunebu	*grandis* (L.) Osbeck
pseudogulgul Hort. ex Shirai	Yoa shaddock	*grandis* (L.) Osbeck
pseudolimon Tan.	False lemon	*limon* (L.) Burm. f.
pseudolimonum Wester	False lemon	*limon* (L.) Burm. f.
pseudopapillaris Tan.	Balanga orange	*aurantium* hybrid
pseudoparadisi Hort. ex Y. Tan.	Uwa pumelo	*paradisi* Macf.
pseudosunki Hort. ex Tan.	Kobeni	*reticulata* Blanco
pyriformis Hassk.	Ponderosa lemon	*limon* (L.) Burm. f.
reshni Hort. ex Tan.	Reshni, Chota-Kitchli	*reticulata* Blanco
reticulata Blanco	Satsuma, mandarin	*reticulata* Blanco
rissoi Risso	Risso's citrus	*limon* (L.) Burm. f.
rokugatsu Hort. ex Y. Tan.	Za-daidai, June sour orange	*aurantium* L.
rugulosa Hort. ex Tan.	At-anni	*paradisi* Macf.
sinensis Osbeck	Sweet orange	*sinensis* (L.) Osbeck
sinograndis Hort. ex Tan.	Oto-mikan	*paradisi* Macf.
southwickii Wester	Southwicks papeda	*celebica* var. *southwickii* (Wester) Swing.

suavissima Hort. ex Tan.	Okan, Unshu-mikkitsu	*reticulata* Blanco
subcompressa Hort. ex Tan.	Sakitsu	*reticulata* Blanco
succosa Hort. ex Tan.	Nidonari, Jimikan	*reticulata* Blanco
sudachi Hort. ex Shirai	Sudachi	*ichangensis* hybrid
suhuiensis Hort. ex Tan.	Szu-ui-kom, Shikaikan	*reticulata* Blanco
suizabon Hort. ex Tan.	Sujabo, Suizabon	*grandis* (L.) Osbeck
sulcata Hort. ex Takahashi	Sanbokan	*paradisi* Macf.
sunki Hort. ex Tan.	Sunki	*reticulata* var. *austera* Swing.
tahibana Tan.	Tachibana orange	*tachibana* (Mak.) Tan.
taiwanica Tan. et Shimada	Nansho-daidai	*aurantium* hybrid
tamurana Hort. ex Tan.	New summer orange, Hyuga-natsumikan	*sinensis* (L.) Osbeck
tangerina Hort. ex Tan.	Dancy	*reticulata* Blanco
tankan Tan.	Honey barrel orange	*reticulata* Blanco
tardiferax Hort. ex Tan.	Mankitau	*reticulata* Blanco
tardiva Hort. ex Shirai	Natsu-mikan, Giri-mikan	*reticulata* Blanco
tarogayo Hort. ex Tan.	Tarogayo	*reticulata* Blanco
temple Hort. ex Y. Tan.	Temple orange	*reticulata* hybrid
tengu Hort. ex Tan.	"Red-faced" Shigetom, Kinkuneba, Tengu-mikan	*paradisi* Macf.
tosa-asahi Hort. ex Y. Tan.	Tosa-asahi	*paradisi* Macf.
truncata Hort. ex Tan.	Jagatara	*grandis* (L.) Osbeck
tumida Hort. ex Tan.	Fukure-mikan	*reticulata* Blanco
ujukitsu Hort. ex Tan.	Bushukan	*sinensis* (L.) Osbeck
unshiu Marc.	Satsuma	*reticulata* Blanco
vitensis Tan.	Moli Kurikuri	*macroptera* hybrid
webberi Wester	Kalpi	*aurantifolia* (Christm.) Swing.
westeri Tan.	Samuyao, small fruit papeda	*micrantha* var. *microptera* Wester
wilsonii Tan.	Ichang lemon	*ichangensis* × *C. grandis* hybrid
yamabuki Hort. ex Y. Tan.	Yamabuki	*paradisi* Macf.
yatsushiro Hort. ex Tan.	Yatsushiro-mikan	*reticulata* Blanco
yuge-hyokan Hort. ex Tan.	Yuge-hyokan	*paradisi* Macf.
yuko Hort. ex Tan.	"Yuko"	*ichangensis* × *C. reticulata* hybrid
zeylanica Tan.	Gondh-huntra	*paradisi* Macf.

GLOSSARY

Bark patch ...... Piece of bark without bud that is inserted in bark in same way as bud—sometimes called blind bud

Blotch .... Chlorotic spot with diffuse, indefinite, or irregular margin

Candidate tree .... Tree to be tested for virus infection

Cheesy .... Consistency of cheese

Dappled .... Spotted as certain animals (ex. Percheron horse)

Index .... Test a plant (citrus) for presence of certain viruses

Indicator plant ... Variety of citrus used for testing purposes by reason of its strong reaction to virus in question

Inverse pit ...... Peglike outgrowth of wood that fits into similarly shaped pit in cambial face of bark

Leaf patch ...... Small piece of leaf inserted under T- or U-shaped cut in bark

Lime test .... Test for tristeza using West Indian lime (*C. aurantifolia* (Christm.) Swing.), also called Key lime, Mexican lime, and baladi lime

Lopsided .... Unsymmetrical

Mild strain ...... Form of virus that induces mild symptoms in standard indicator plant

Peg .... Toothlike outgrowth from cambial face of bark that fits into pit in wood

Pit (collectively, pitting) Depression in cambial face of wood, varying from less than 1 mm. to several centimeters, often elongated in direction of branch elongation

Take .... Union of bud, graft, or other tissue with budded tree

T-bud .... Bud inserted into T-shaped cut in bark (T may be inverted)

☆GPO: 1967—O 263—337